

Noscomial aspergillosis : state of the art

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Size of aspergillosis problem globally

1. Invasive aspergillosis ~ 4,000 cases/year in UK [60M population], >5M at risk in EU
2. Chronic pulmonary aspergillosis - ~3M cases prevalence
3. Cystic fibrosis - >9,000 cases [15%+ *Aspergillus* infection or allergy of ~60,000 CF cases]
4. Asthma 197M in adults, of which ~10-20% severe, UK and USA have very high prevalence rates
5. ABPA in asthma - ~3M worldwide (2.1% of adults with asthma)
6. Severe Asthma with Fungal Sensitisation (SAFS) - ~6M worldwide (33% of 10% (severe only))

Invasive mould infections in Austria

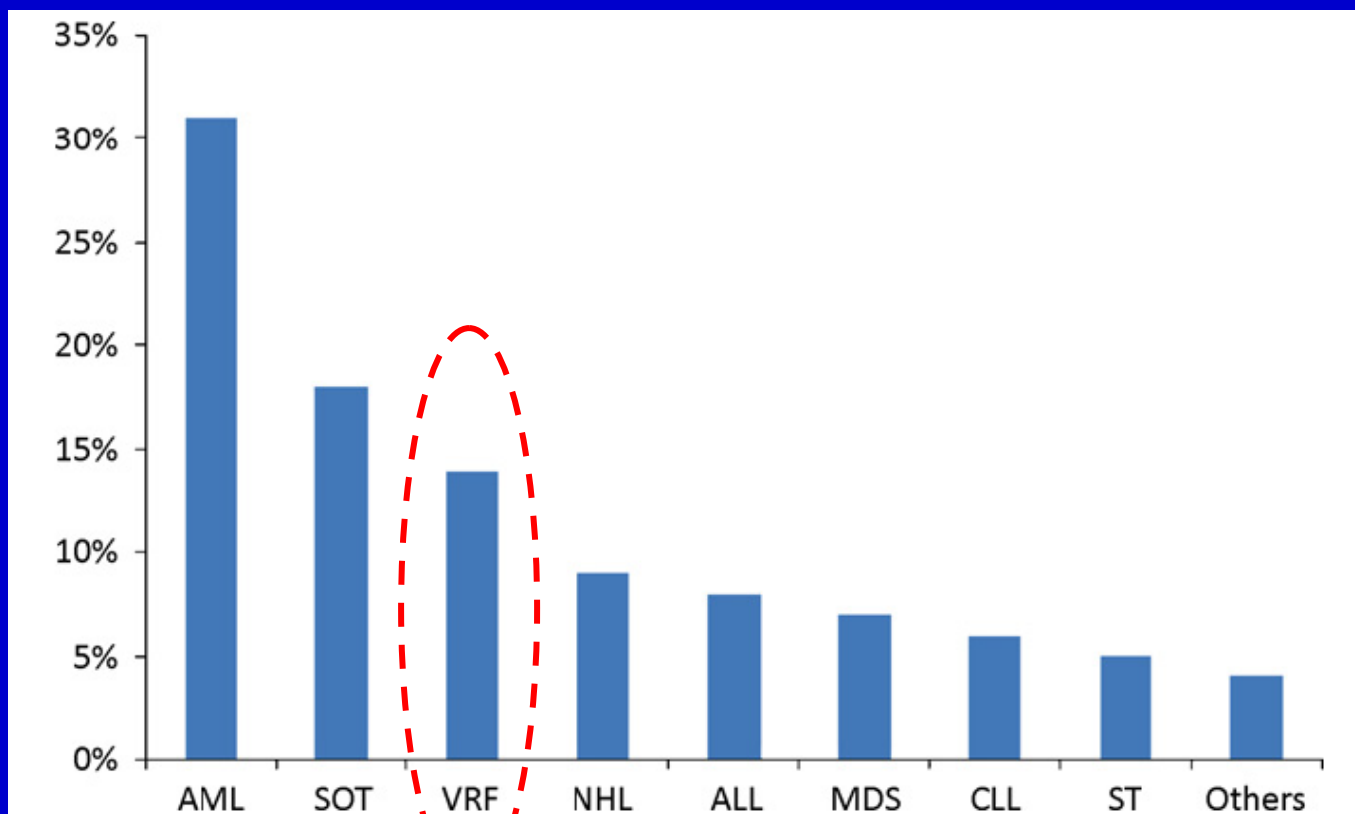
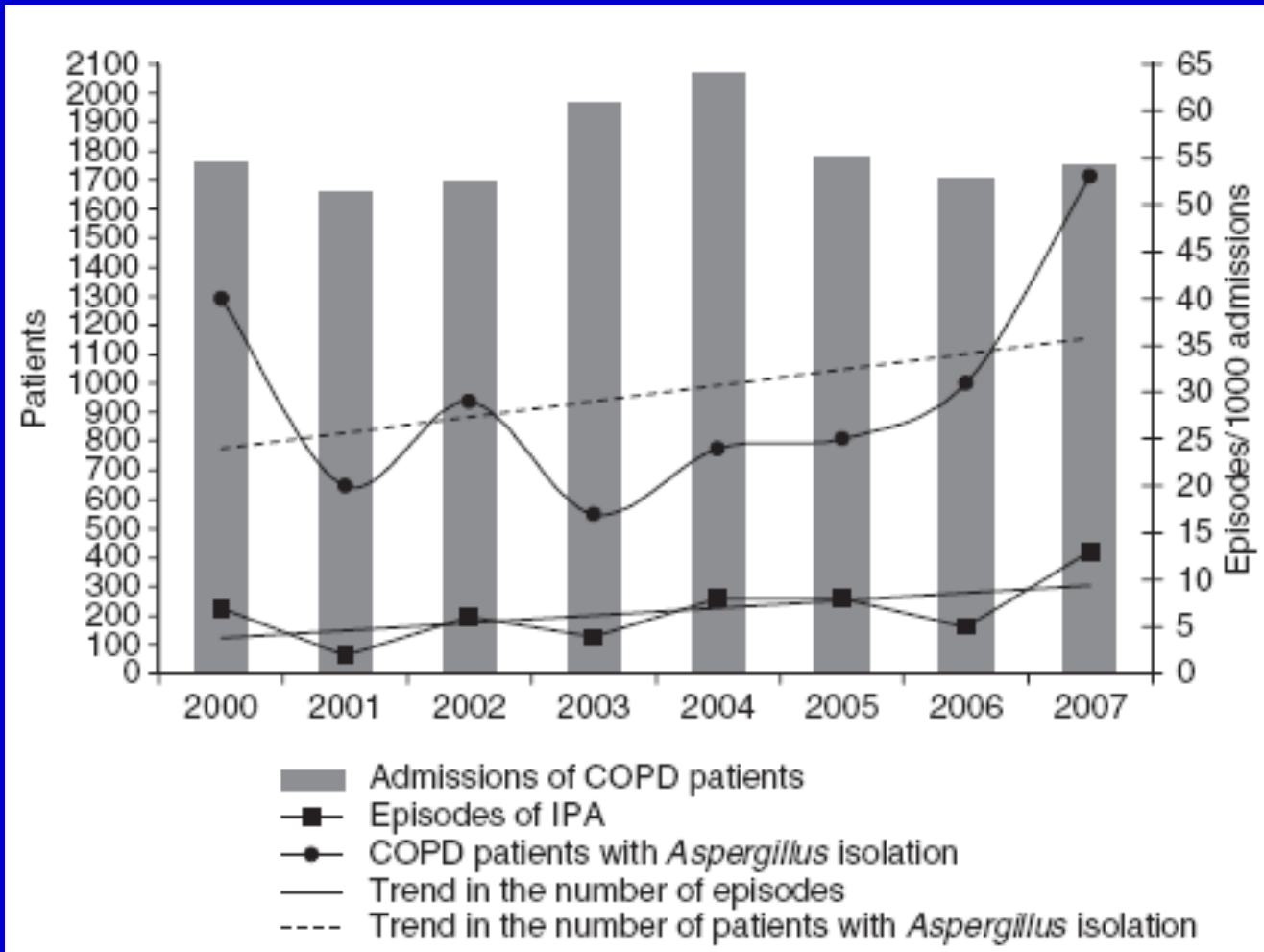


Fig. 1. Overview of underlying diseases in 186 patients with invasive mould infections. AML, acute myelogenous leukaemia; SOT, single-organ transplantation; VRF, various risk factors (mainly Intensive Care Unit patients without underlying haematological malignancies); NHL, non-Hodgkin's lymphoma; ALL, acute lymphatic leukaemia; MDS, myelodysplastic syndrome; CLL, chronic lymphatic leukaemia; ST, solid tumour.

Aspergillus, IPA and COPD



~ 22% of *Aspergillus* in COPD = invasive aspergillosis

Aspergillus, IPA and COPD

	Wald	p	OR
ICU admission	4.758	0.029	2.406
Chronic heart failure	3.649	0.056	2.102
Accumulated dose of corticosteroids prior to admission ^a	6.213	0.013	2.987
Accumulated dose of corticosteroids during admission ^b	13.338	0.000	4.568
Antibiotic treatment ^a	5.924	0.015	2.570
Constant	66.327	0.000	0.034

ICU, intensive-care unit.

^aIn the 3 months prior to admission.

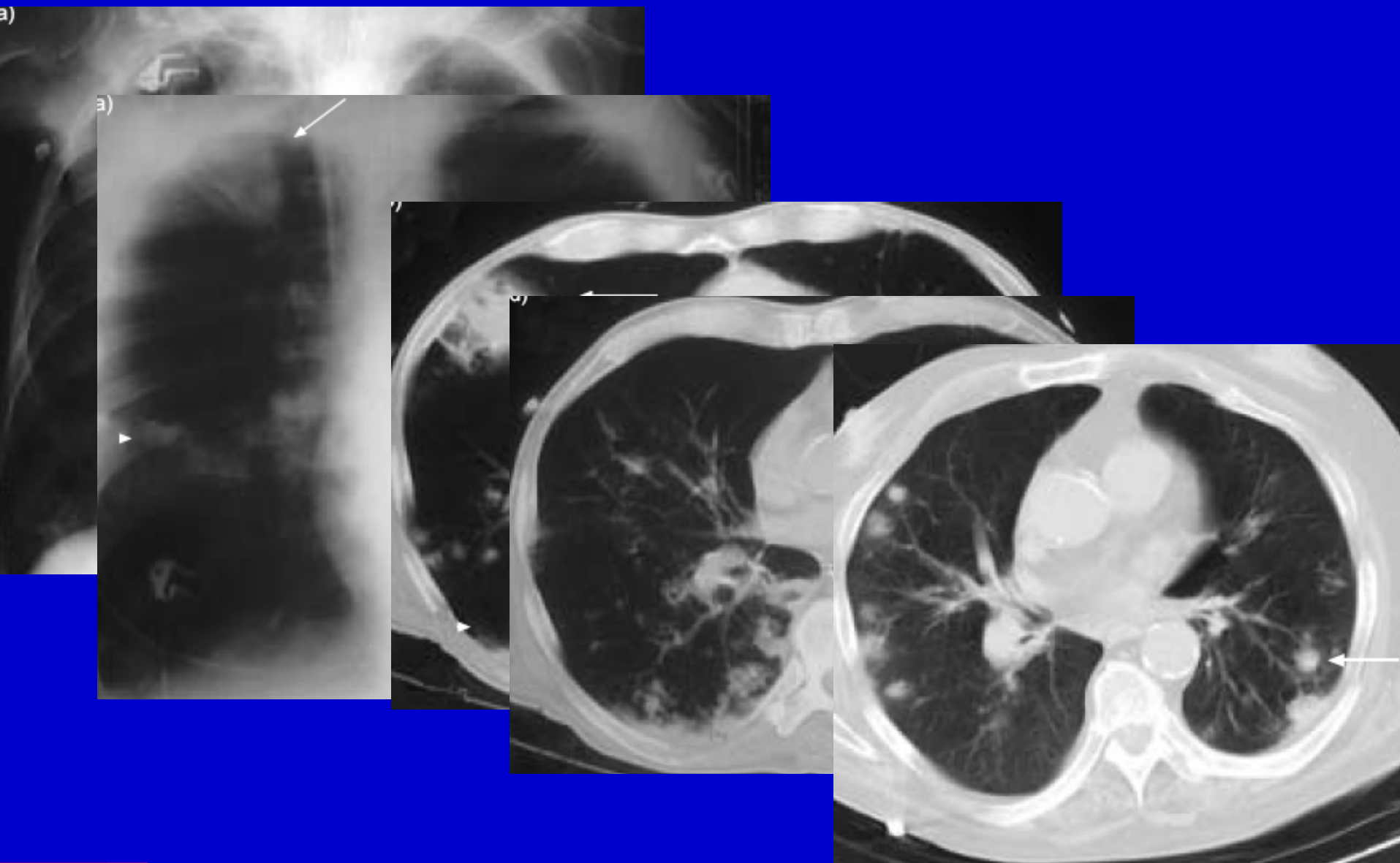
^bFrom admission to the first clinical isolation of *Aspergillus* from LRT samples.

Aspergillus, IPA and COPD

Clues to the diagnosis of IA

- GOLD stage 3 or 4.
- Excess wheezing (consider tracheobronchitis)
- Worsening infiltrates in an 'exacerbation' (66%)
- Bilateral infiltrates (55%)
- Culture of Aspergillus
- High corticosteroid exposure recently
- Do NOT expect fever (38%), chest pain or haemoptysis

Invasive aspergillosis in COPD



Invasive aspergillosis in ICU

127 of 1850 (6.9%) consecutive medical ICU admissions with IA or colonisation (micro/histol).

89/127 (70%) did not have haematological malignancy

67/89 proven/probable IA, 33 of 67 (50%)
COPD

Risk factors for invasive aspergillosis in ICU

Table 2. Risk of invasive aspergillosis among patients admitted to the intensive care unit (ICU; medical, mixed or surgical).

High-risk category

Neutropenia (neutrophil count, <500 neutrophils/mm³)

Hematological malignancy

Allogeneic bone marrow transplantation

Intermediate-risk category

Prolonged treatment with corticosteroids before admission to the ICU

Autologous bone marrow transplantation

Chronic obstructive pulmonary disease

Liver cirrhosis with a duration of stay in the ICU >7 days

Solid-organ cancer

HIV infection

Lung transplantation

Systemic diseases requiring immunosuppressive therapy

Low-risk category

Severe burns

Other solid-organ transplant recipients (e.g., heart, kidney, or liver transplant recipients)

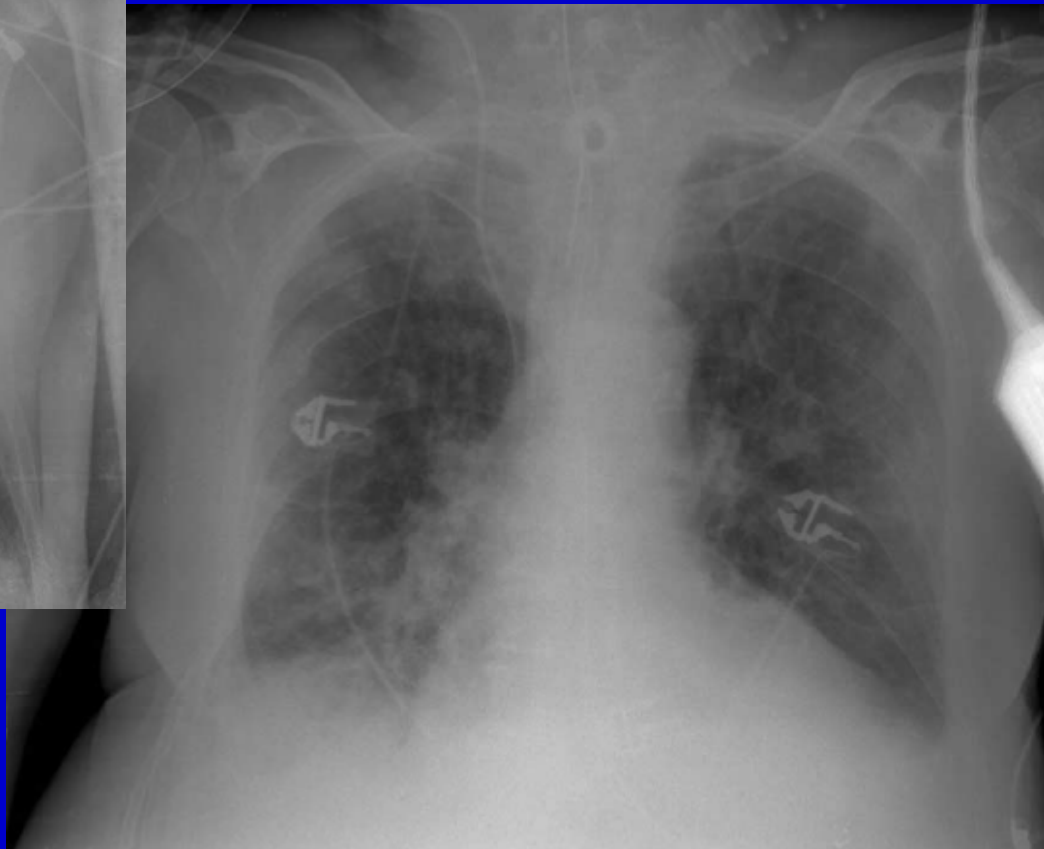
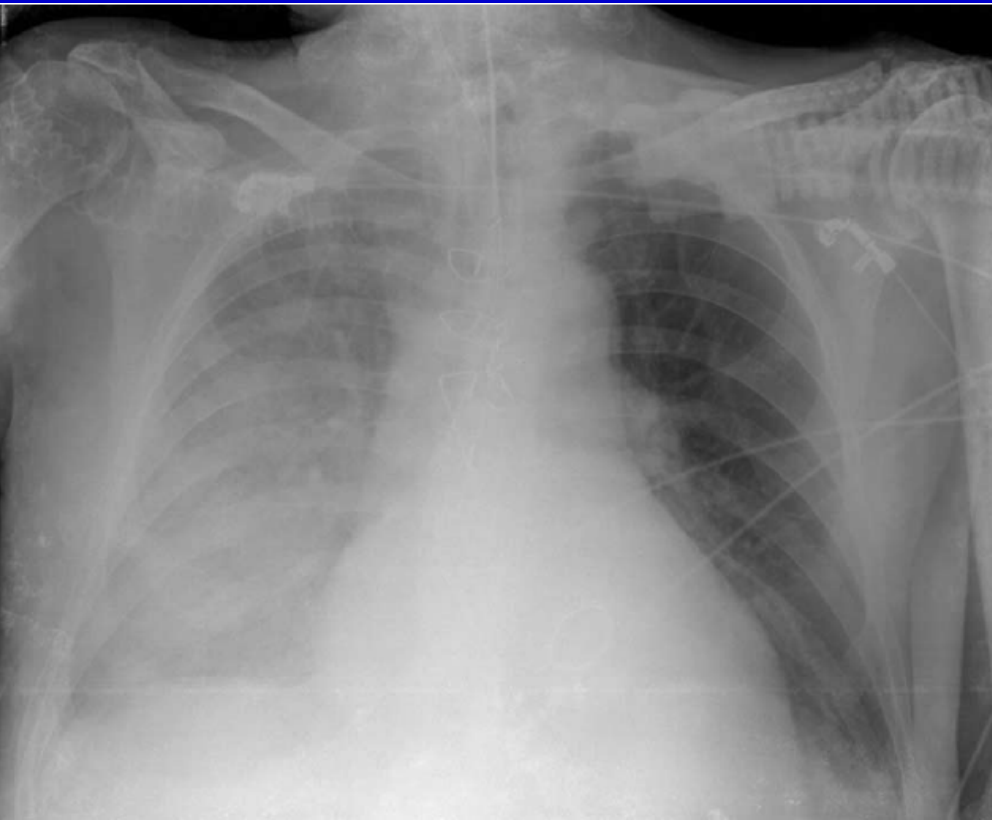
Steroid treatment with a duration of ≤ 7 days

Prolonged stay in the ICU (>21 days)

Malnutrition

Post-cardiac surgery status

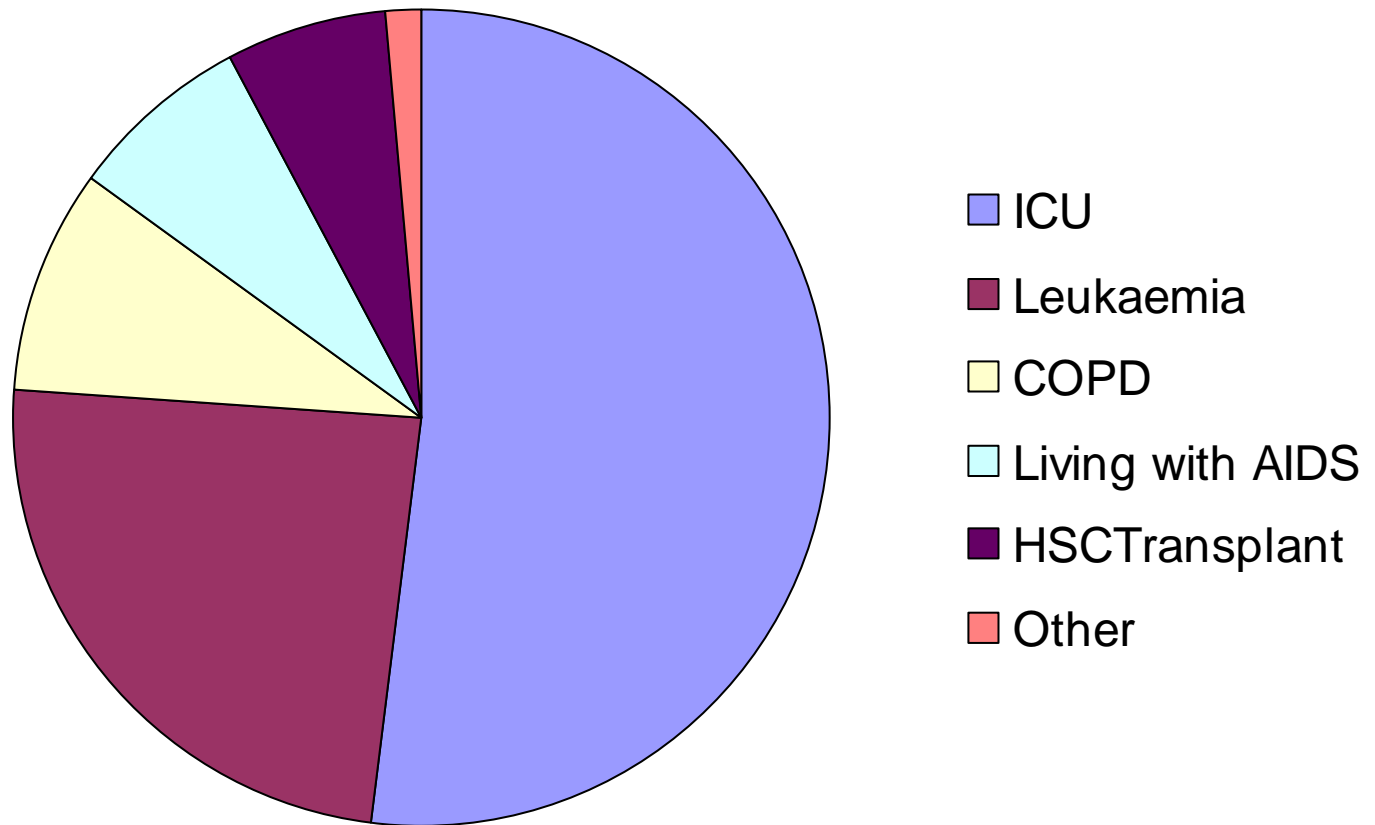
Radiology completely unhelpful in suspecting the diagnosis



Distribution of IA cases

Risk Groups

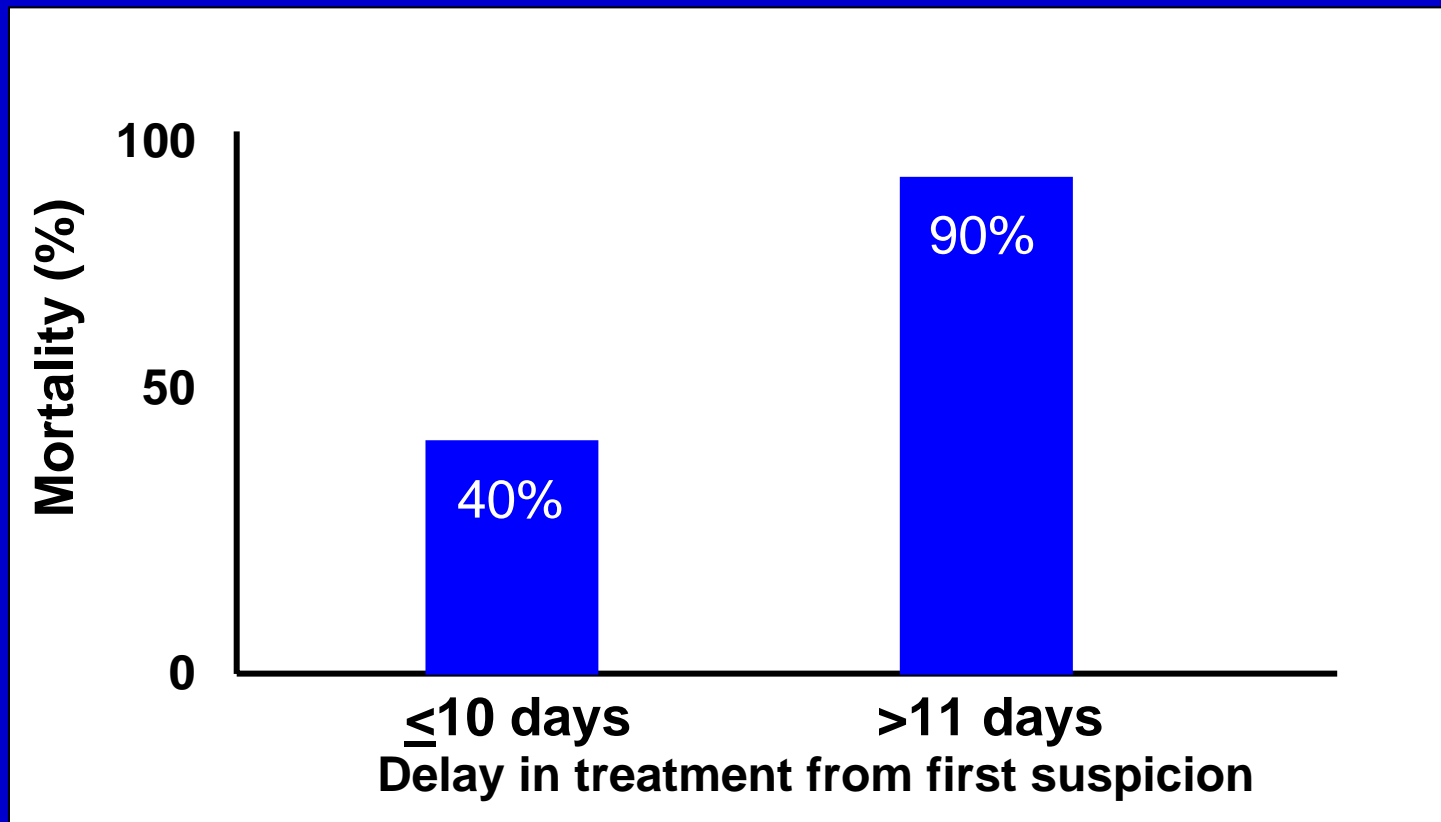
Prediction based on cases numbers and risk



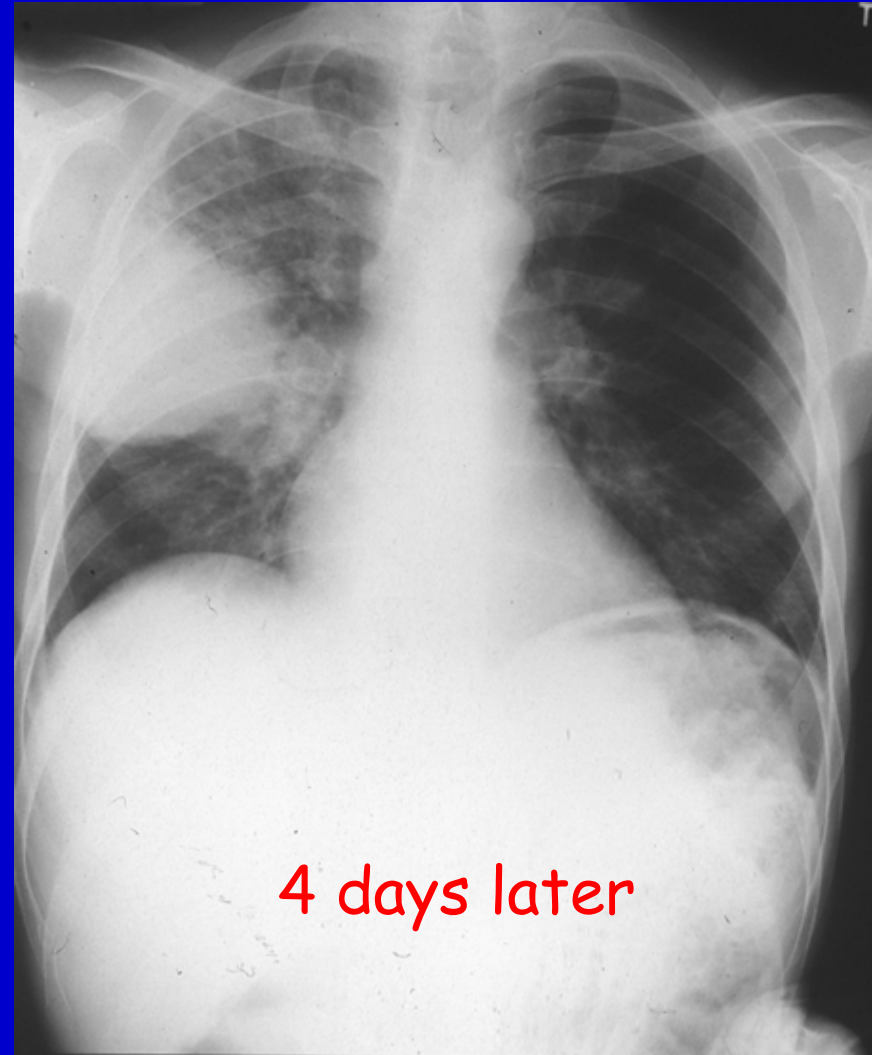
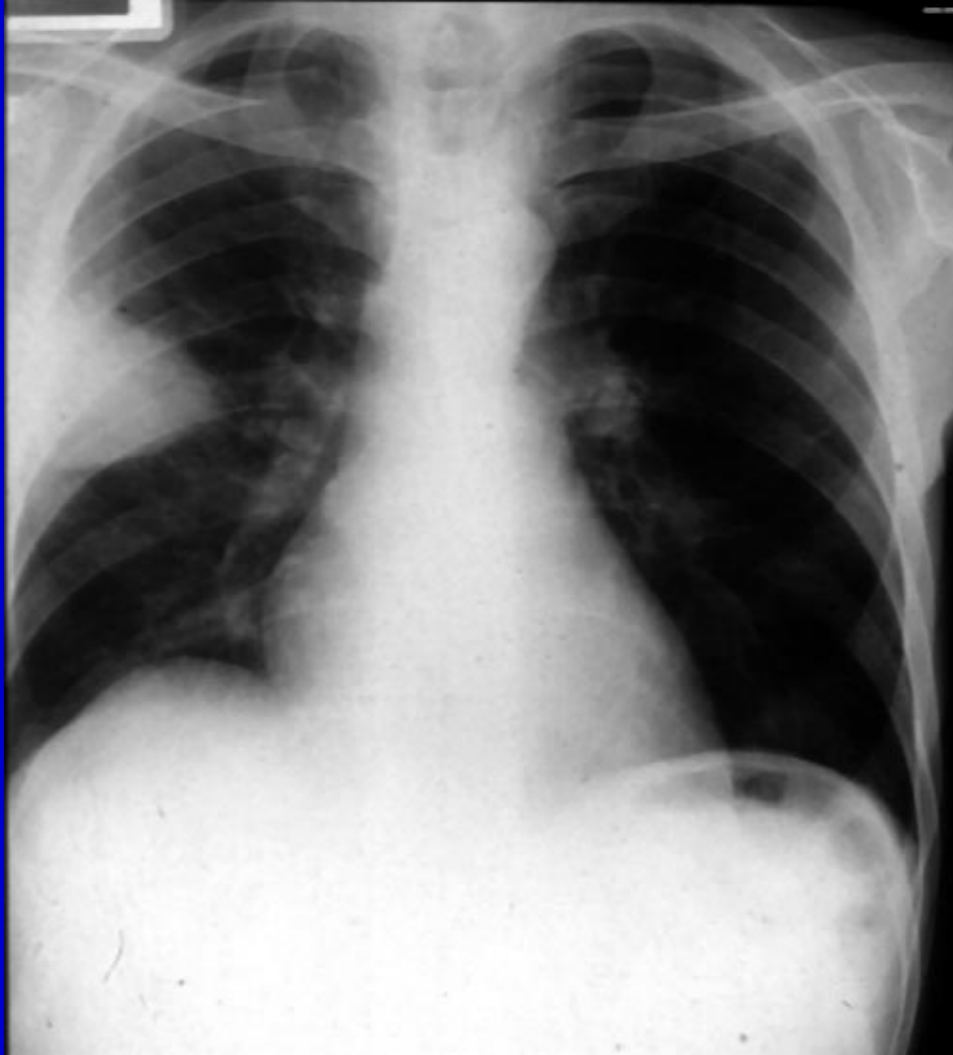
Limitations of current diagnostics for aspergillosis

- a) slow
- b) insensitive
- c) imprecise (species, resistance)

Early diagnosis of invasive aspergillosis is very important for survival

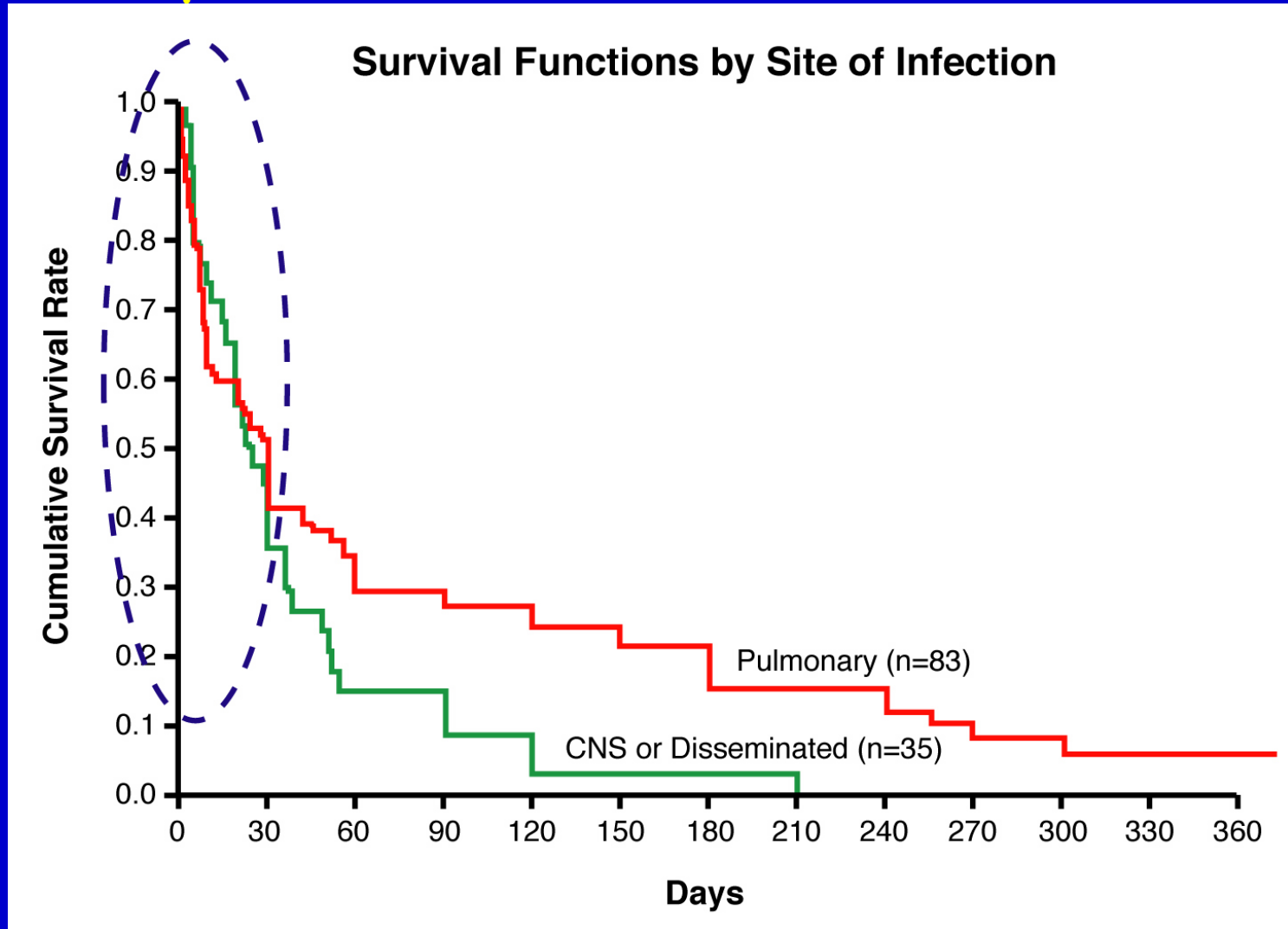


Pace of progression of IPA

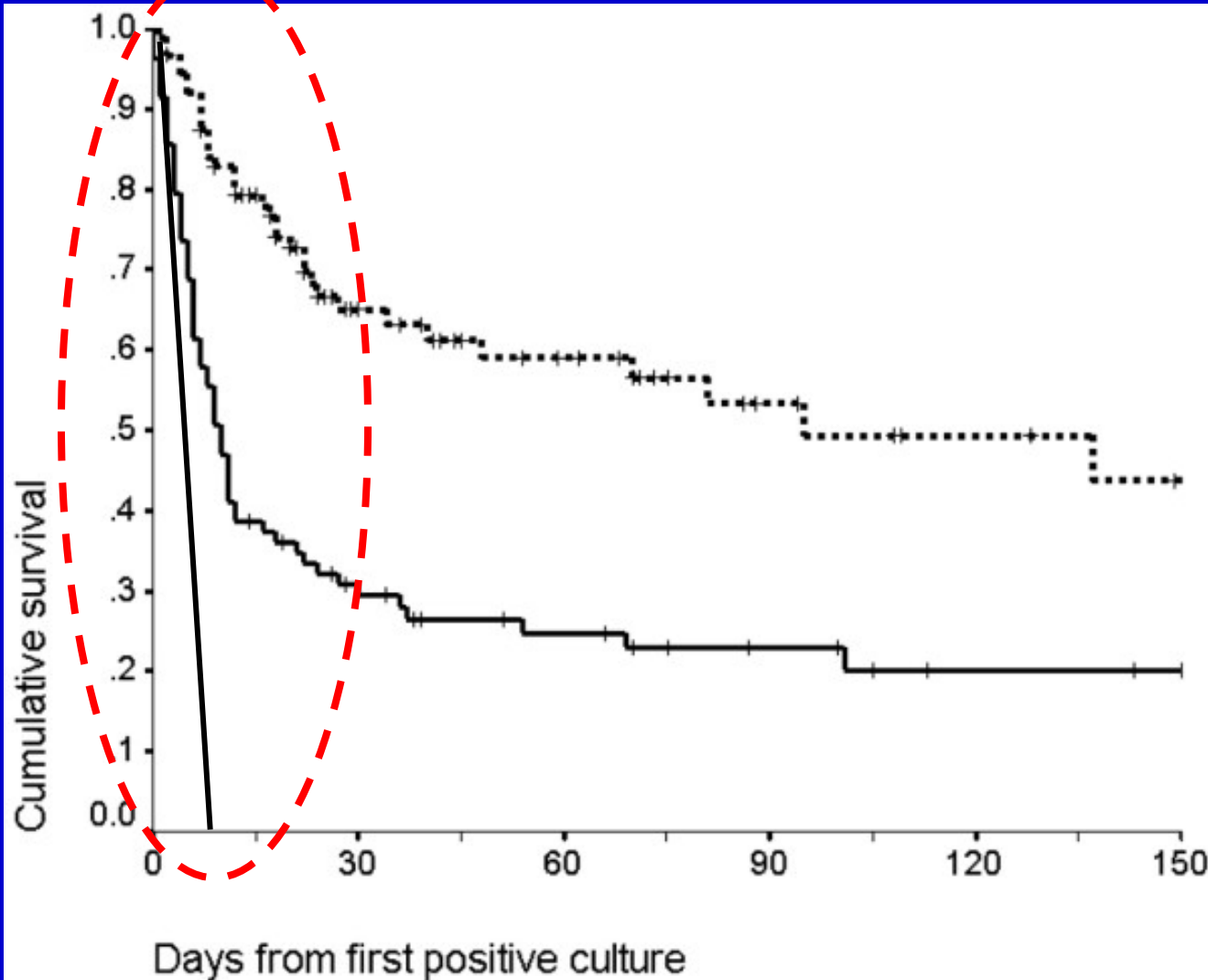


4 days later

Survival from invasive aspergillosis [Amphotericin B + itraconazole era]



Invasive aspergillosis in ICU



Aspergillus detected,
no infection
N = 89

Invasive aspergillosis
+ treatment
N = 73

Invasive aspergillosis
no treatment
N = 12

Limitations of current diagnostics for aspergillosis

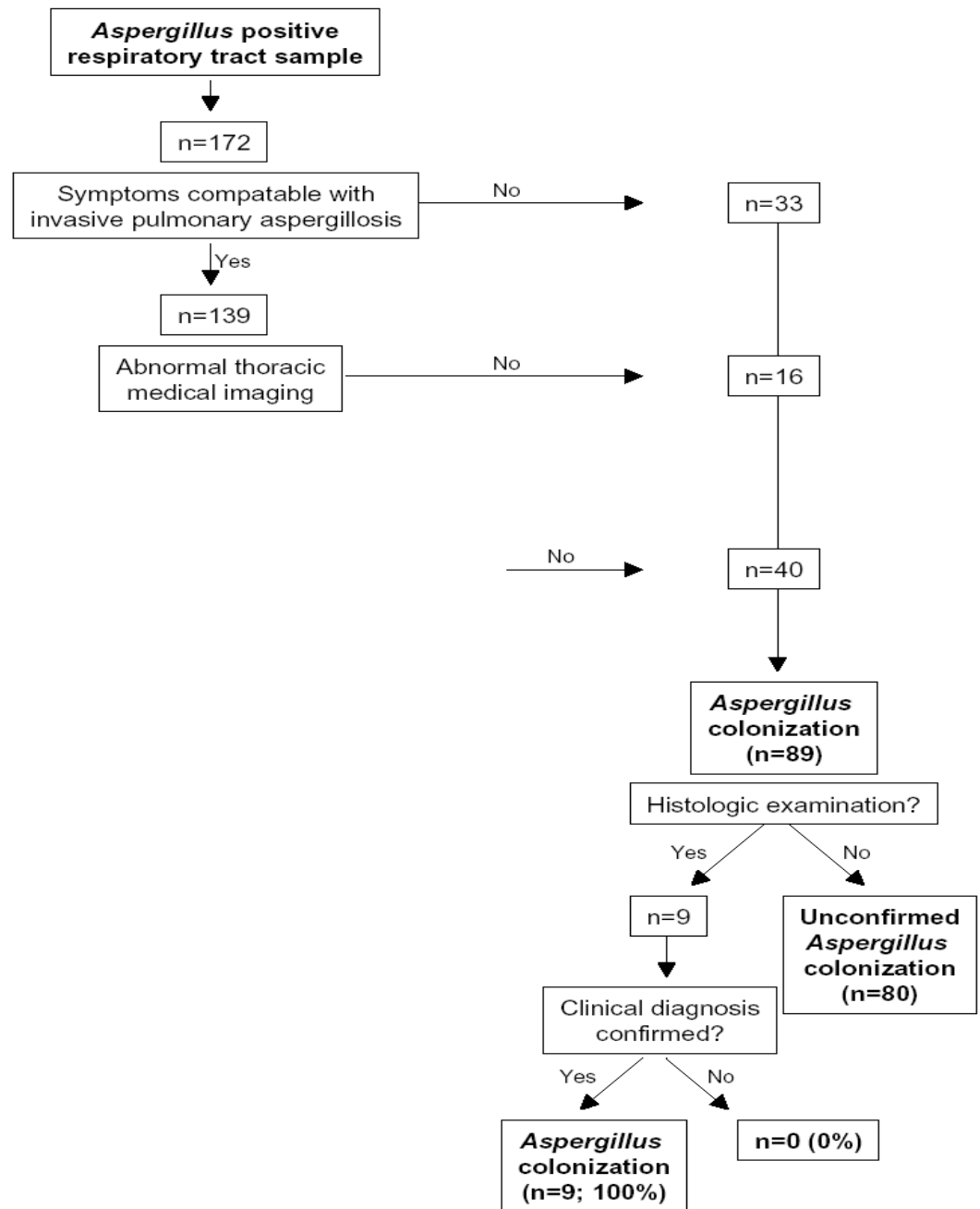
- a) slow
- b) insensitive
- c) imprecise (species, resistance)

IA in non-neutropenic patients in Switzerland

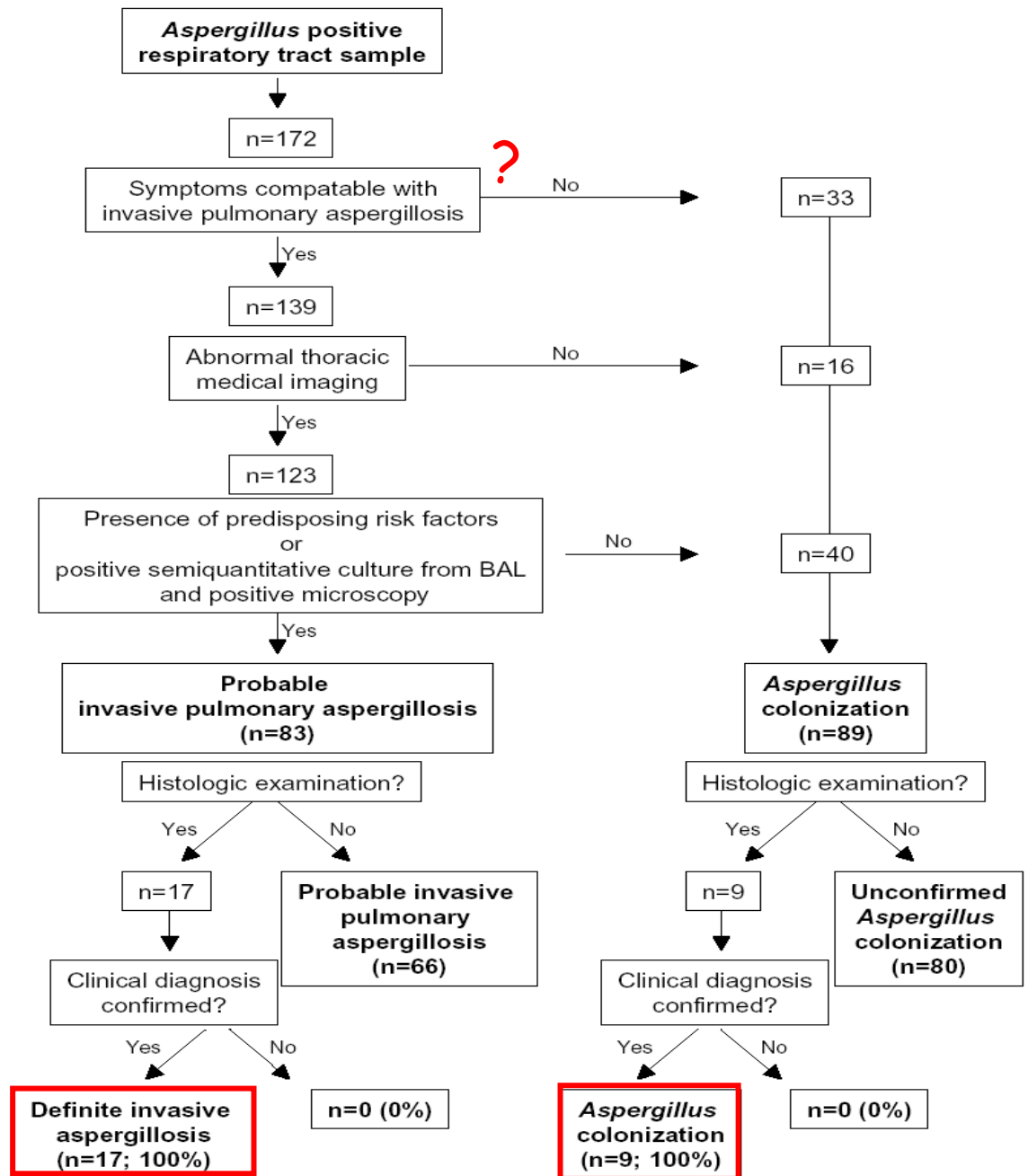
Variable	IA diagnosis post-mortem N=16		IA diagnosis ante-mortem N=26		OR
	n	%	n	%	
Age (median, IQR)	65	57-71	58	48-63	2.1‡
Male gender	13	81.3	19	73.1	0.6
Cancer	7	43.8	6	23.1	2.6
Transplantation	3	18.8	10	38.5	0.4
Chronic lung disease	5	31.3	15	57.7	0.3
ICU stay	6	37.5	12	46.2	0.7
Mechanical ventilation	6	37.5	8	30.8	1.4
Prednisone	12	75.0	12	46.2	3.5
Immunosuppression	4	25.0	7	26.9	0.9
CRP mg/μl (median, IQR)	74	22-187	20	0-183	1.0†

‡per 10 years older
†per 10 mg/ml increase in CRP

Respiratory samples +ve for *Aspergillus* in ICU



Respiratory samples +ve for *Aspergillus* in ICU



Aspergillus Antigen in BAL in ICU

- 110 patients out of 1109 ICU admissions assessed
- 26 proven IA cases
- Sensitivity and specificity of BAL GM detection was 88% and 87%
- 11 of 26 (42%) BAL culture positive
- Serum GM negative in 100%

Aspergillus Antigen in BAL

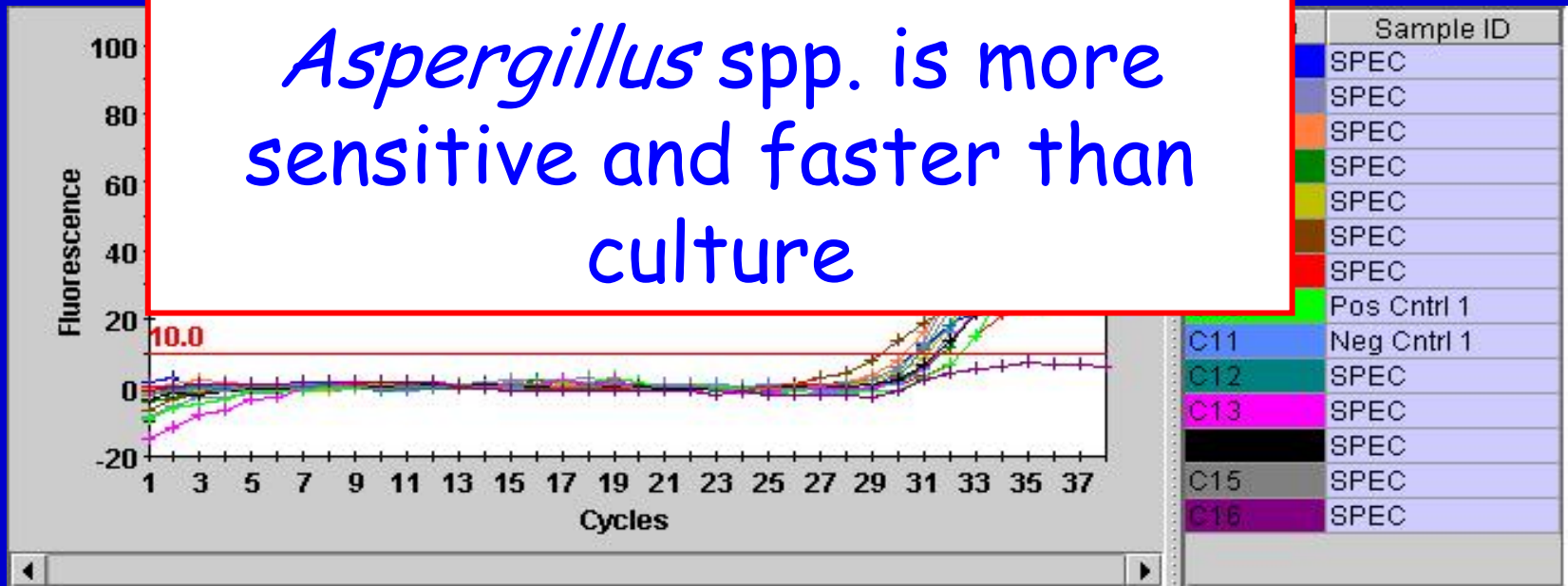
- 13/17 (76%) in acute leukaemia with CT abnormality
- 20/20 (100%) in haem-onc pts with IPA
- 37/49 (76%) in HSCT & haem-onc with IPA
- 6 of 11 (55%) immunocompromised (8 of 11 +ve by PCR)
- 5/20 (25%) in suspected IFIs

- 17/17 (100%) in neutropenic patients before antifungal Rx,
0% after 3d antifungal therapy

Real-time PCR for *Aspergillus* spp.



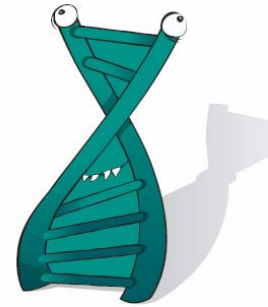
Real-time PCR for *Aspergillus* spp. is more sensitive and faster than culture



Products

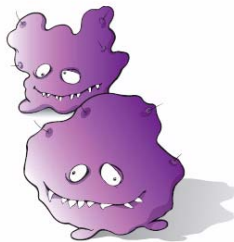
MycXtra[®]
DNA extraction

Effective extraction
of fungal DNA
from clinical
respiratory samples



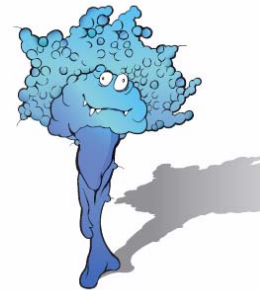
MycAssay[™]
Pneumocystis

Rapid detection of
Pneumocystis DNA from
lower respiratory tract
samples using
Real-Time PCR



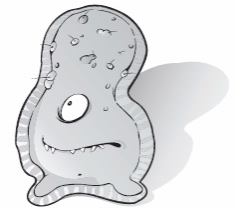
MycAssay[™]
Aspergillus

Rapid detection of
Aspergillus DNA from
lower respiratory tract
samples using
Real-Time PCR



MycArray[™]
Yeast ID

Species identification
of Candida and
other yeasts

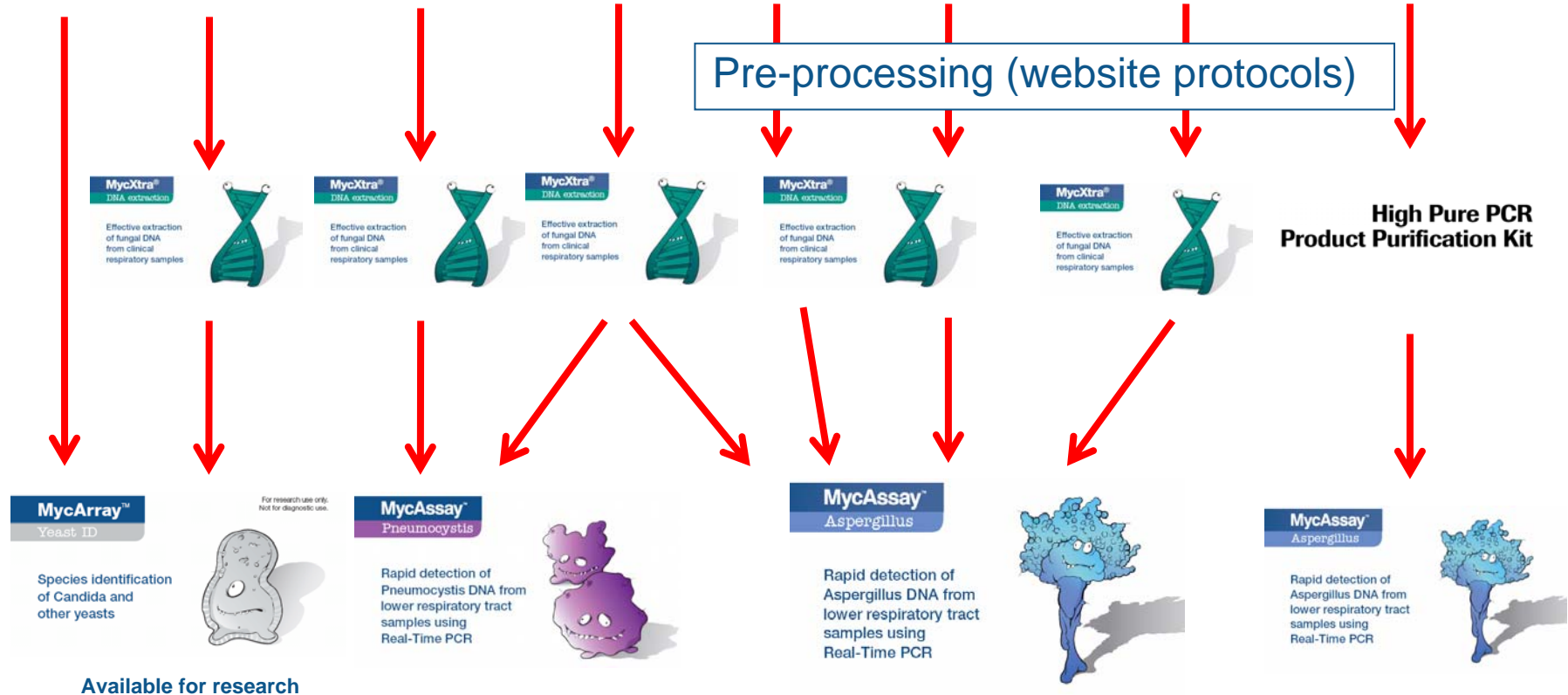


For research use only.
Not for diagnostic use.

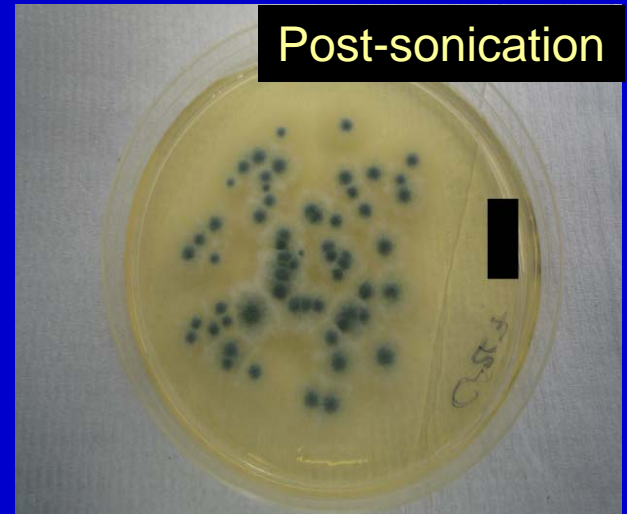
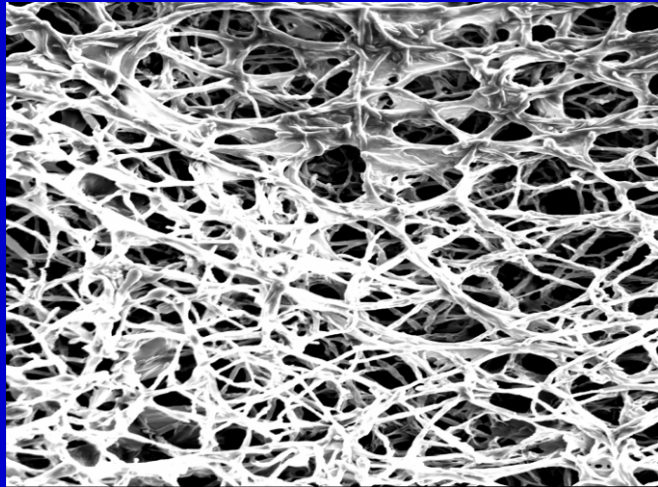
Available for research

Samples that can be processed

Culture Induced sputum BAL Sputum CF sputum Tissue Serum
 Blood culture



CF and sputum culture



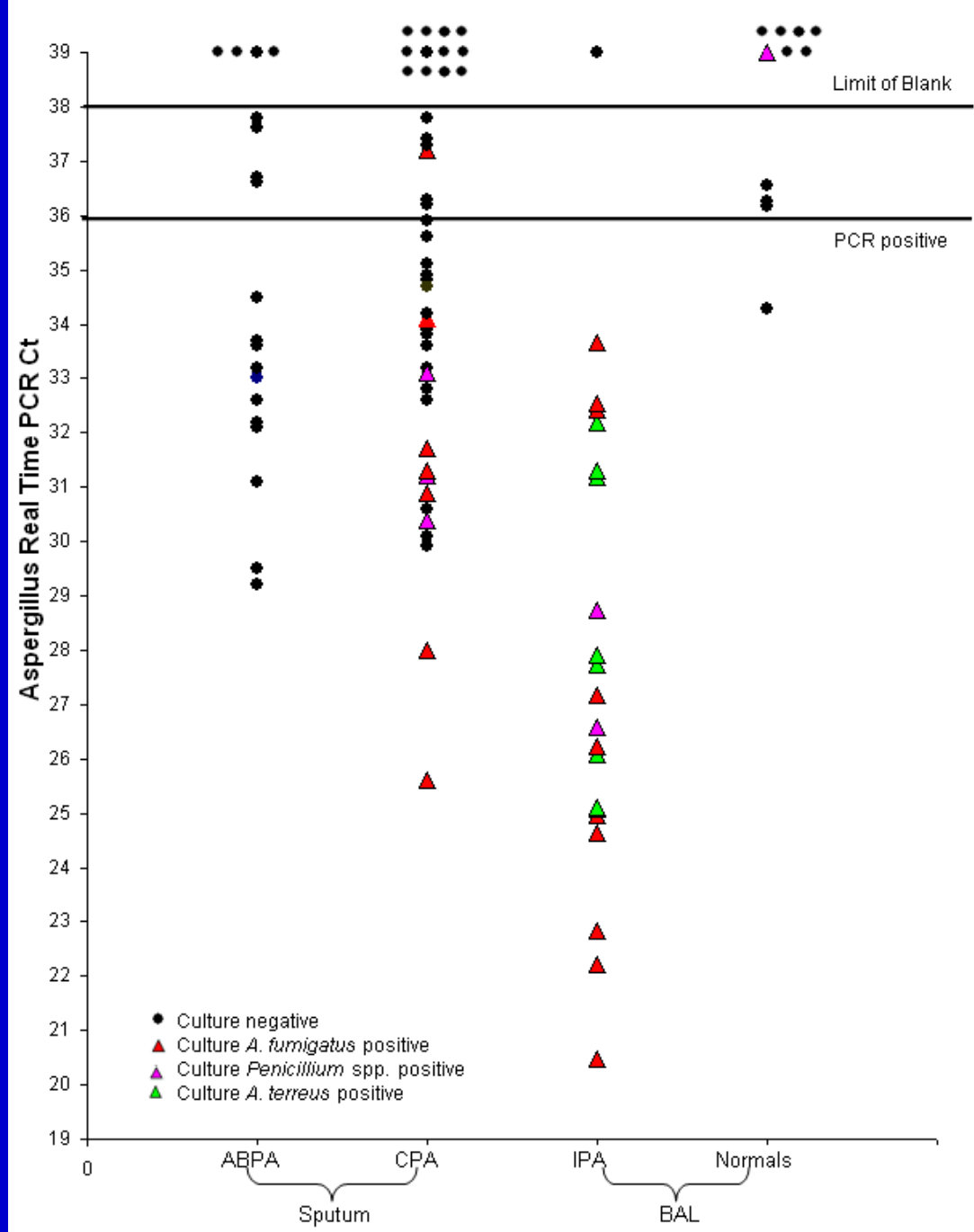
Processing CF sputum for culture and PCR
- with dithiothreitol at 37°C for 30 mins and
sonication

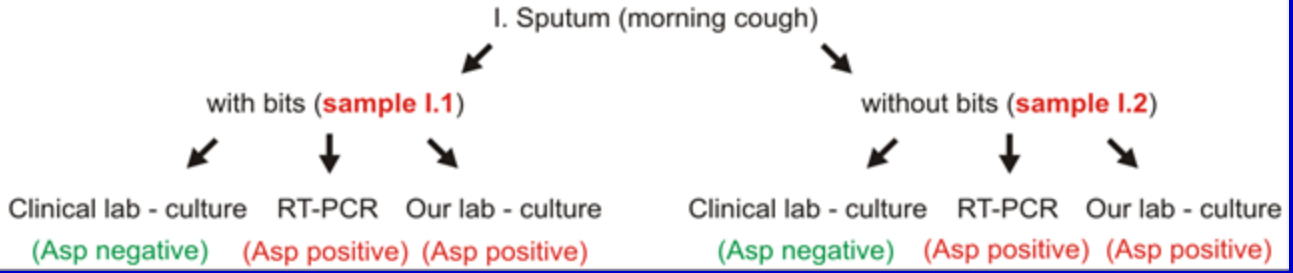
	PCR Positive	PCR Negative
Culture Positive	33	0
Culture Negative	53	31

PCR is 260% more sensitive than culture; $p < 0.0001$

Detection of *Aspergillus* in respiratory fluids is superior to culture

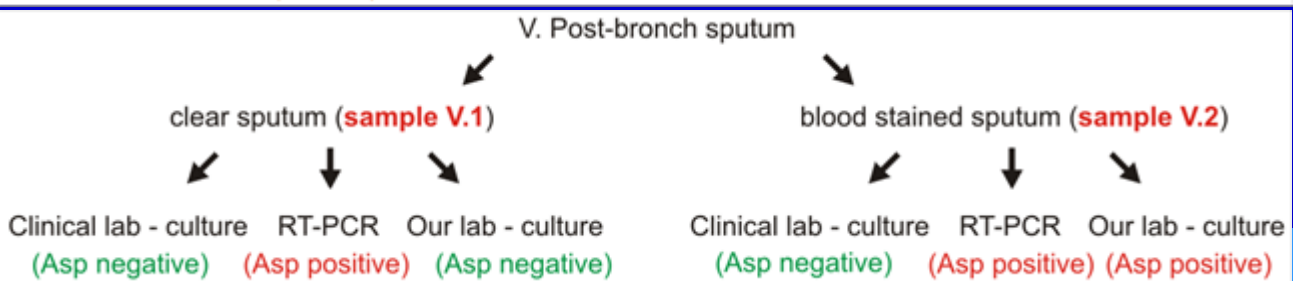
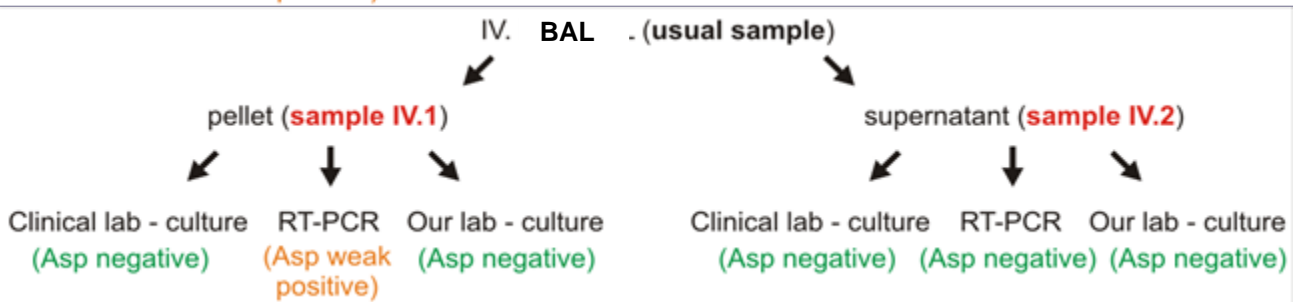
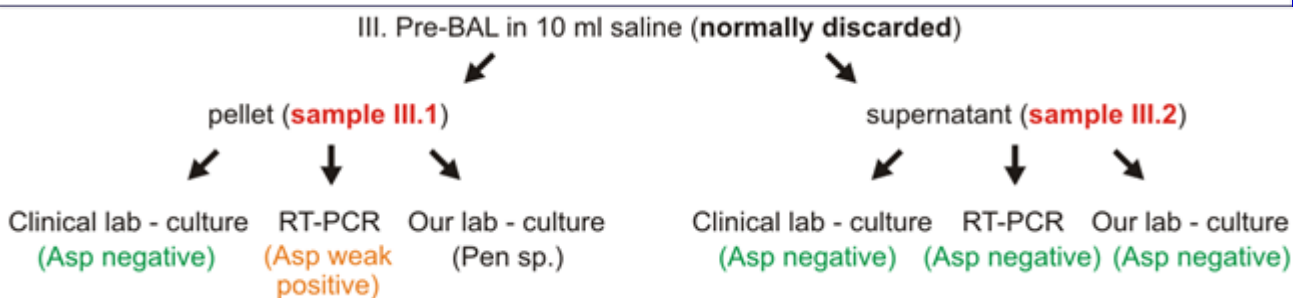
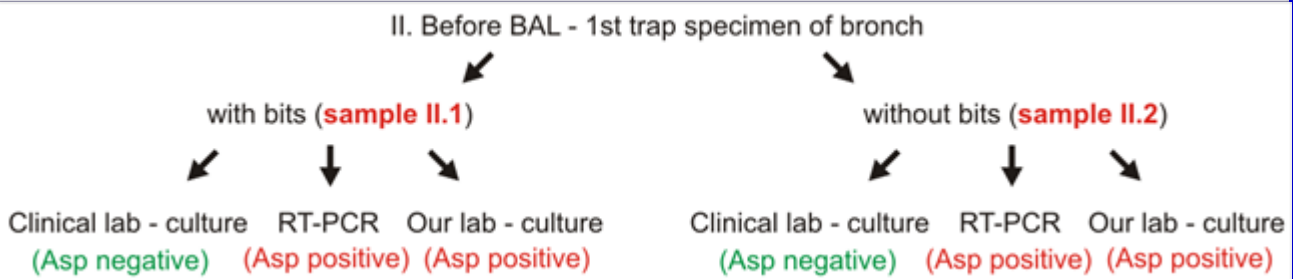
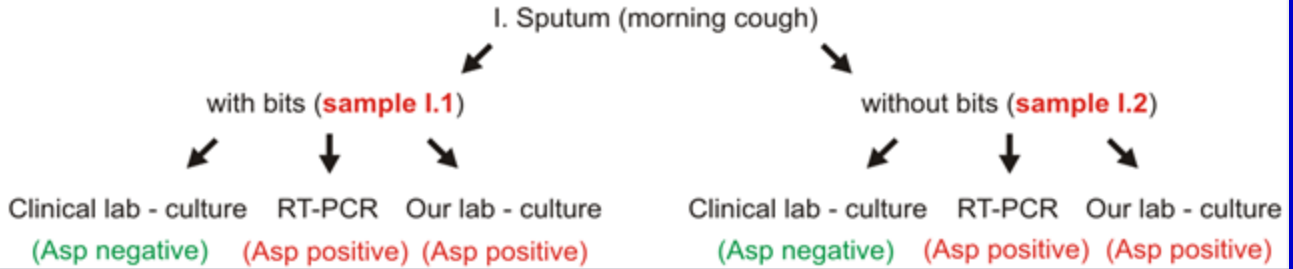
DNA can be used for direct resistance detection





Bronchoscopy in an ABPA patient on no treatment





Bronchoscopy in an ABPA patient on no treatment

Abundant mixed inflammatory cells with ciliated columnar cells and a few fungal hyphae, in keeping with *Aspergillus*. A few Charcot leyden crystals. No malignant cells.

Sputum and BAL currently processed using BSOP57

Sputum

Equal volume of Sputasol (dithiothreitol 1.4%)

Mix

1uL

[Oup

Need for a reappraisal of culture methods for fungi in respiratory samples

BAL

Pelle

on 2

or replace with PCR

- plated

Role of Aspergillus PCR in BAL/sputum

Given a typical sensitivity and specificity, and a **high** prevalence, a **positive** predictive value is more useful than the **negative** predictive value

MycAssay Aspergillus - Respiratory IFU

Sensitivity 94%
Specificity 91%

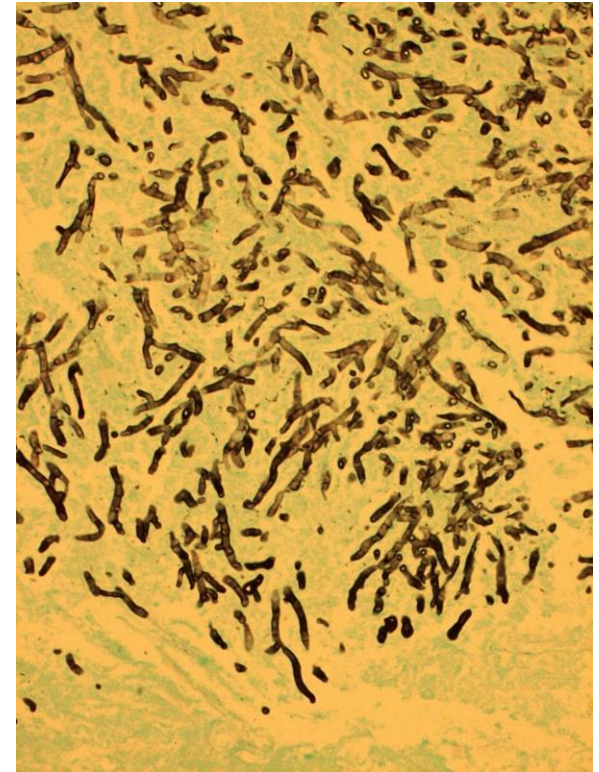
	Prevalence			
	1%	10%	20%	40%
PPV	9%	54%	72%	87%
NPV	100%	99%	98%	96%

Confirmatory test

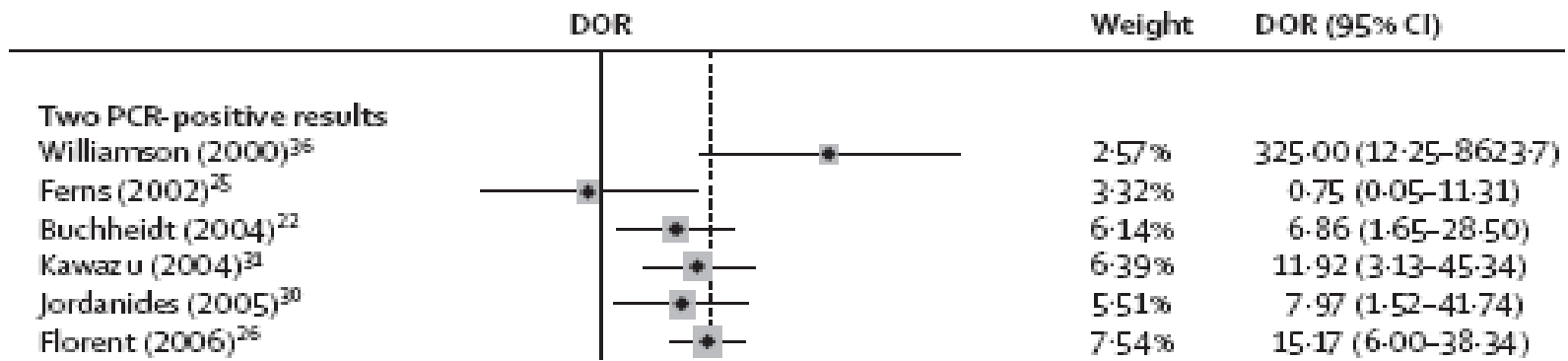
MycAssay™ Aspergillus

Paraffin-embedded tissue PCR

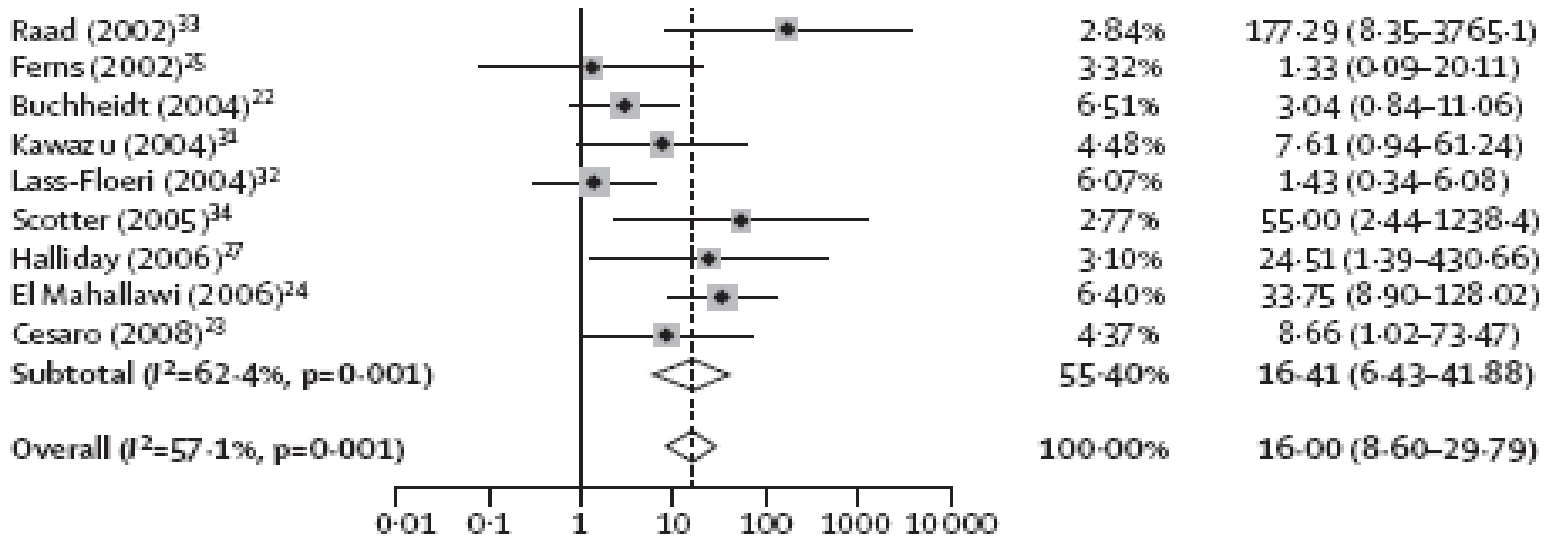
- 50 tissue samples from 50 patients
 - Hyphae typical of *Aspergillus*
 - Deparaffinization of tissue sections with xylene and methanol
 - MycXtra DNA extraction
 - Phenol/chloroform purification
 - MycAssay real time PCR
 - Confirmation by ITS sequencing of *A. fumigatus*
-
- All 50 had a positive PCR signal with Cts from 23.5 – 32.1



Chander et al, submitted for publication



PCR diagnosis of IA in blood Meta-analysis



MycAssay™ Aspergillus: Analytical credibility

Table 1. Real-time PCR detection of varying concentrations freshly extracted

Aspergillus fumigatus DNA

Dilution	DNA concentration (genomes μl^{-1})	Reaction input (genomes) ^a	Reaction input (rRNA copies) ^b	MycAssay (Ct value)	In-House (Ct value)
Neat	12000	120000	6360000	17.8	17.9
-1	1200	12000	636000	22.1	22.4
-2	120	1200	63600	26.0	26.1
-3	12	120	6360	29.3	29.8
-4	1.2	12	636	32.1	33.5
-5	0.12	1.2	63.6	34.5	37.3

^a10 μl input volume

^bBased on the mean number of rRNA copies being 53 per genome (Herrera *et al.* 2009)

White L. et al, Manuscript submitted

MycAssay™ Aspergillus: Analytical Credibility

Species (Input per reaction)	MycAssay™ <i>Aspergillus</i>	“In-house” PCR
<i>A. fumigatus</i> (250-10 ⁶ copies)	21.6 to 32.7 cycles	23.7 to 35.7 cycles
<i>A. fumigatus</i> (5000 copies)	29.7 cycles	32.2 cycles
<i>A. flavus</i> (5000 copies)	27.7 cycles	30.3 cycles
<i>A. terreus</i> (5000 copies)	29.6 cycles	34.5 cycles
<i>A. niger</i> (5000 copies)	31.1 cycles	36.0 cycles
<i>A. nidulans</i> (5000 copies)	29.1 cycles	33.5 cycles
<i>A. versicolor</i> (5000 copies)	31.0 cycles	36.2 cycles
<i>A. sclerotiorum</i> (5000 copies)	27.0 cycles	34.4 cycles
<i>A. glaucus</i> (5000 copies)	29.3 cycles	45.0 cycles



White L. et al, Manuscript submitted

Diagnosis of IPA in leukaemia using blood PCR

130 haematology patients

Itraconazole prophylaxis for AML and HSCT

Fluconazole prophylaxis for others (ALL, lymphoma etc)

EORTC/MSG criteria applied

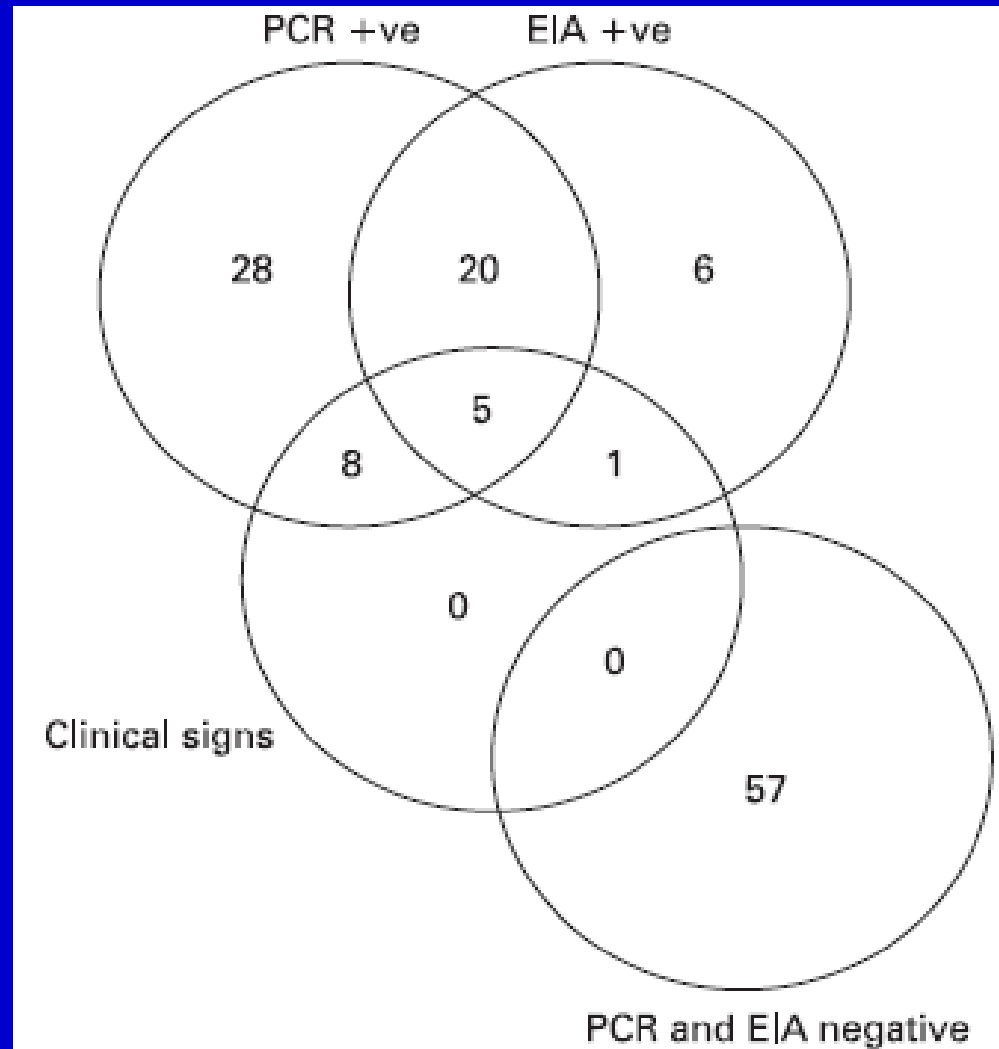
2x weekly sampling

	Sensitivity (%)	Specificity (%)	Positive likelihood ratio*	Negative likelihood ratio	Diagnostic odds ratio†
Single non-reproducible positive PCR result	87.5	98	2.7	0.18	15
Single reproducible positive PCR result			3.8	0.18	21.1
Multiple positive PCR results	75	99	8.3	0.27	30.7

*Likelihood of a positive result in a patient with proven/probable disease versus positive result in a patient without evidence of disease.

†Positive likelihood ratio /negative likelihood ratio.

Diagnosis of IPA in leukaemia using blood PCR



PCR detects disease early: indicator of IFI & earlier than GM for patients on prophylaxis

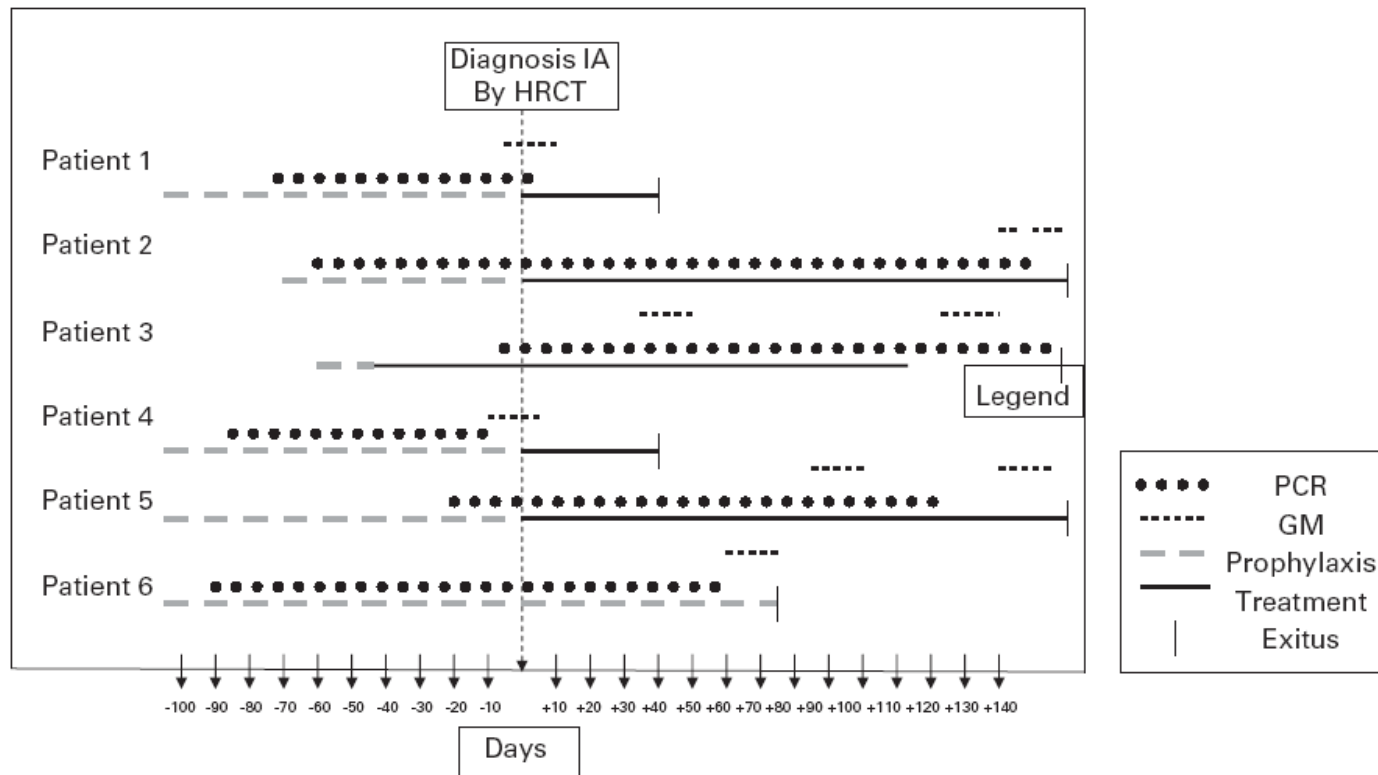


Figure 1 Persistently detected DNAemia in a series of six patients diagnosed with IA. GM = galactomannan; HRCT = high resolution computed tomography; IA = invasive aspergillosis; PCR = polymerase chain reaction.

Role of Aspergillus PCR in serum

Given a typical sensitivity and specificity, and a **low** prevalence, a **negative** predictive value is more useful than the **positive** predictive value

MycAssay Aspergillus - Serum (White paper) multiple positive

Sensitivity **60%**
Specificity **100%**

	Prevalence			
	1%	10%	20%	40%
PPV	100%	100%	100%	100%
NPV	100%	96%	91%	79%

Screening test

Diagnostic approach to IPA in leukaemia using blood PCR

Strategy 1 - screen all patients with serum/blood PCR +/- GM

Strategy 2 - screen only febrile patients with serum/blood PCR +/- GM

Strategy 3 - CT scan + bronchoscopy in those with pulmonary features / persistent fever

Benefits of screening with serum PCR, with high negative predictive value

- Fewer missed diagnoses
- Faster diagnosis with better outcomes
- Reduced cost of prophylactic antifungal therapy

Saving of ~€000's/patient/per year to hospital

- Patient not taking unnecessary medication

Reduce likelihood of fungal resistance being developed

Reduce adverse events and potential for drug interactions

Antifungal therapy of invasive aspergillosis

Treatment



Open study of invasive aspergillosis with caspofungin as primary therapy

61 pts with chemotherapy or auto HSCT received
Caspofungin 70 then 50mg IV daily

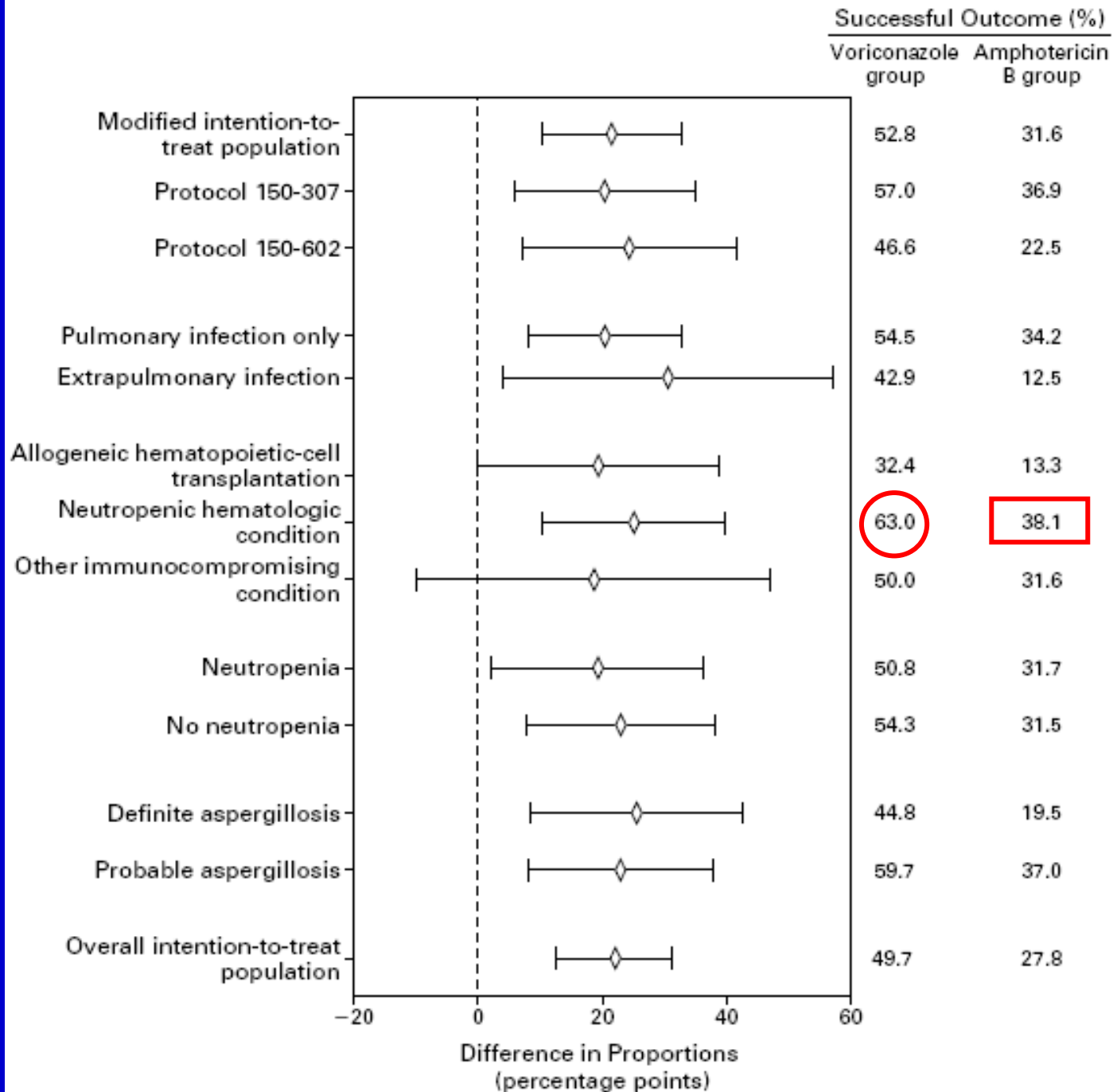
Response	MITT population (N= 61)	
	n	% (95% CI)
Complete	1	2 (0-9)
Partial	19	31 (20-44)
Stable disease	9	15 (7-26)
Disease progression	31	51 (38-64)
Not evaluable ^a	1	2 (0-9)

^aPatient refused treatment.

} 33% response rate

Neutropenia at enrolment (not assessable in one case)		
no	5/9 (56)	0.14
yes	15/51 (29)	

Survival by day 84 = 33/61 (54%)



Open study of invasive aspergillosis with caspofungin as primary therapy

42 pts with allo HSCT , 24 eligible,
Rx Caspofungin 70 then 50mg IV /d

Unrelated donors in 16 patients; acute or chronic GVHD was present in 15, 12 patients were neutropenic (<500) at baseline,

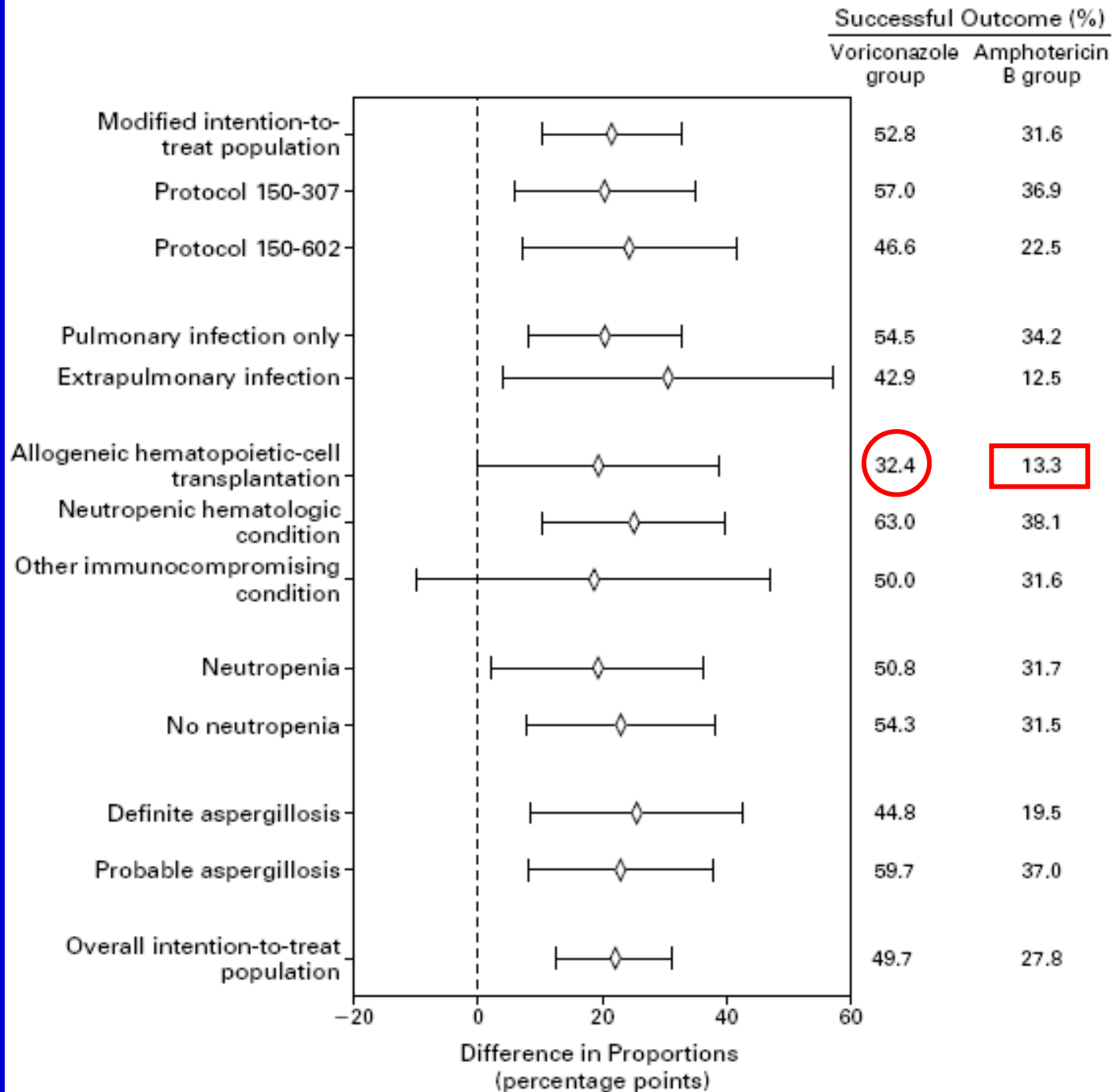
Median duration of caspofungin treatment was 24 days.

At EOT, 10 (42%) had complete or partial response,

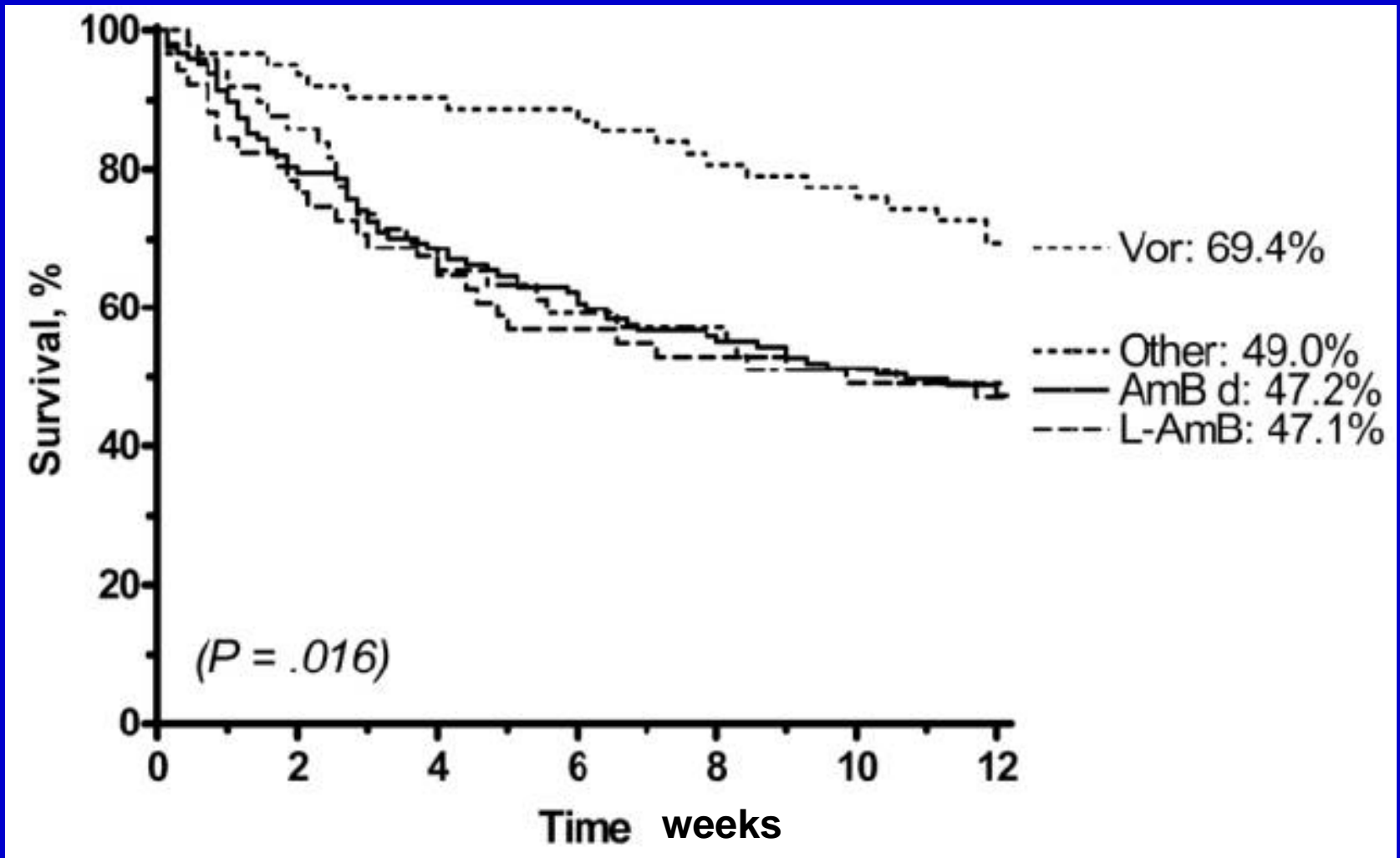
12 (50%) had progressing disease.

At 12 wks, 8 patients (33%) had complete or partial response.

Survival rates at week 6 and 12 were 79 and 50%, respectively.



Impact of voriconazole in real life



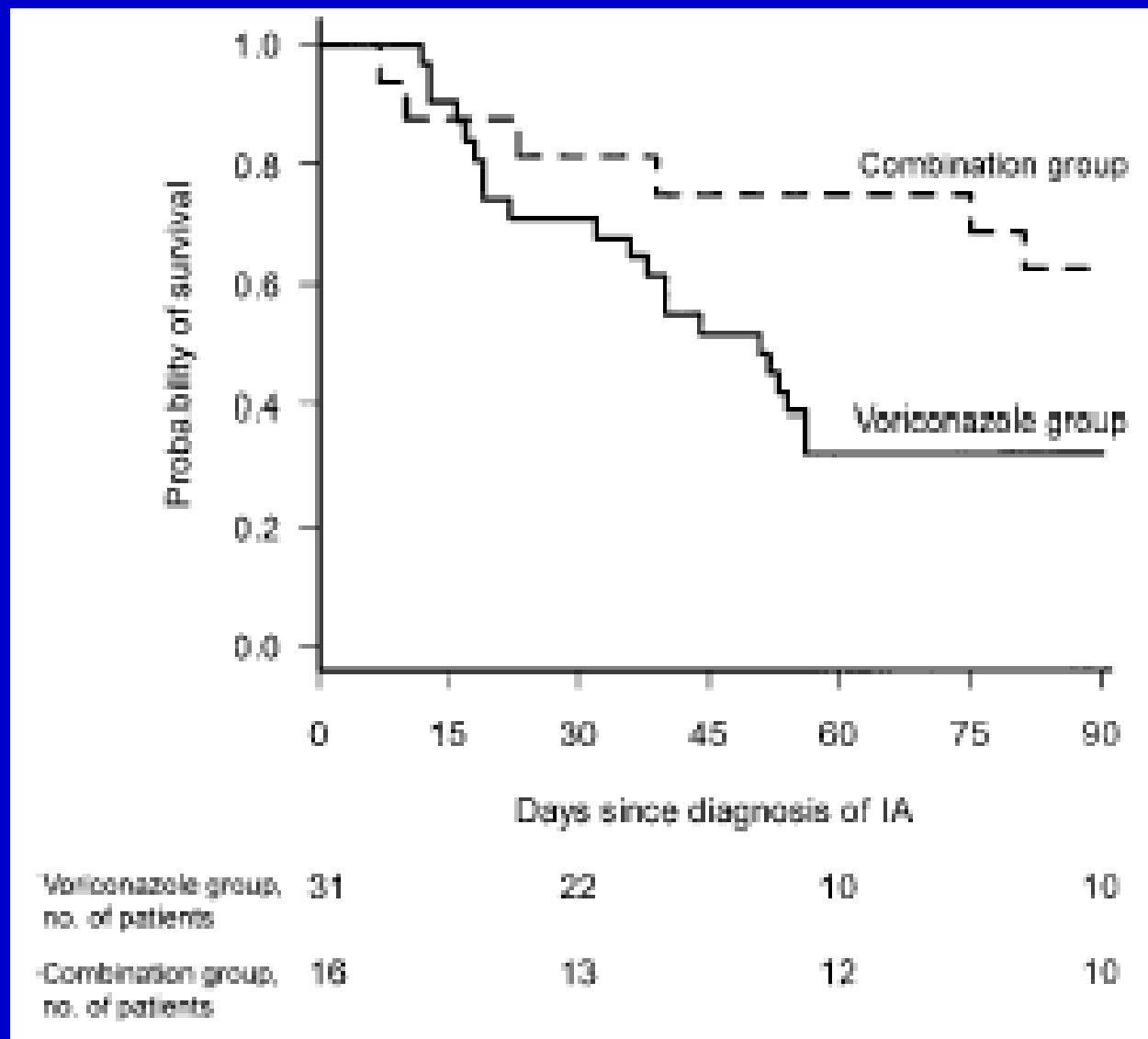
Combination therapy

Combination therapy - invasive aspergillosis

Retrospective
AmB failures
Most HSCT
30/47 proven IA

Multivariate analysis
 $P=0.008$ for
combination and
survival

Curves came together
later



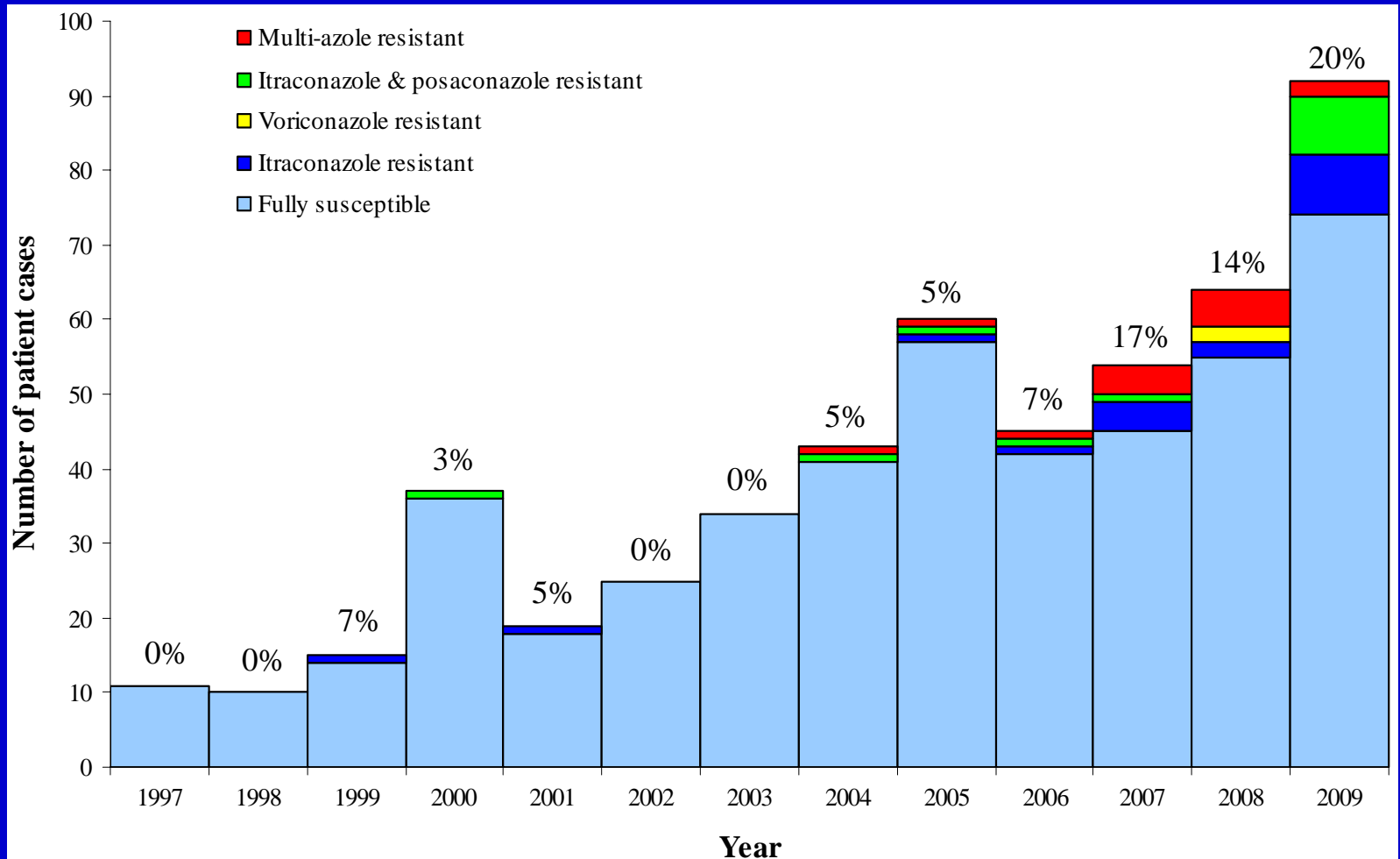
Combination therapy - invasive aspergillosis

Large retrospective series showed no benefit of AmB or voriconazole combination with echinocandin

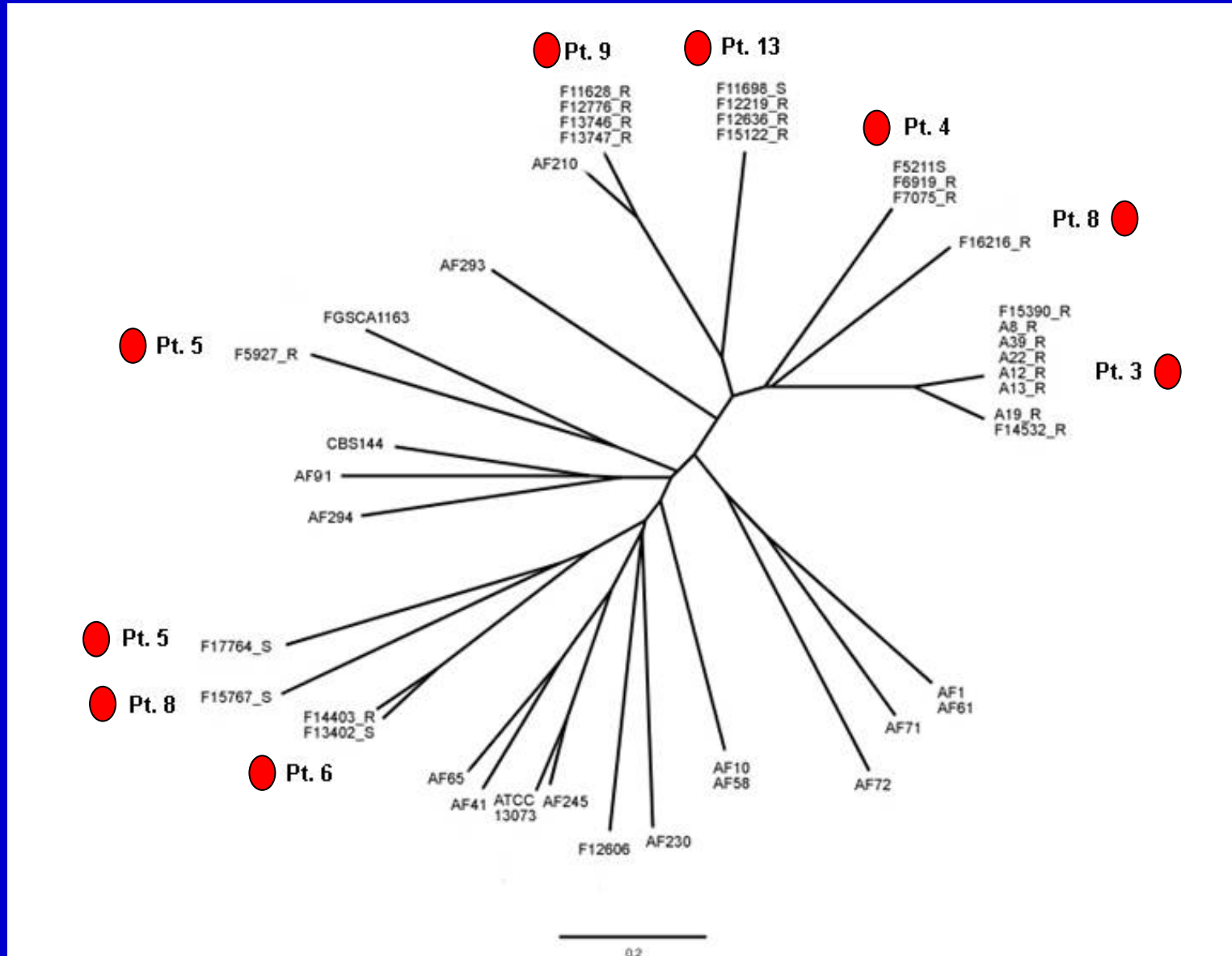
Prospective RCT in progress comparing voriconazole and anidulafungin with voriconazole alone

Antifungal resistance

Azole resistance in *A. fumigatus* in Manchester 1997-2009



Typing of itraconazole resistance in *A. fumigatus*



Azole resistance in *A. fumigatus* in US

California

Itraconazole resistance 13 of 25 (52%) in 2002-2009,
compared with 13 of 126 (10%) in 1987-2001

Detroit

Triazole resistance in 18 of 37 (49%) in 2009,
compared to 11 of 45 (24%) in 2003

Costs of IA

- In 1996, per case cost in US was \$62,500 per hospital stay so cost to the USA was ~ \$674M
- In 1998, per case cost was \$72,792: \$36,867 was extra hospitalization cost attributable to IA
- In 2000, in children IA cost \$49,309 compared with immunocompromised children without IA \$9,035
- In 2003 in Netherland, IA cost ~€32,651 (voriconazole)
- In 1998-2005 in Turkey, each case of IA cost US\$49,336, of which 96% was the cost of drugs

Conclusions

- Corticosteroid Rx and critical illness are significant risk factors for IA
- Many cases still not diagnosed until after death, perhaps majority
- PCR is more sensitive than culture in respiratory fluids, tissue biopsy and blood
- Serum PCR may be positive early and in spite of antifungal prophylaxis in haematology patients
- Voriconazole remains the antifungal agent of choice
- Azole resistance an increasing problem



The Aspergillus Website



www.aspergillus.org.uk



The Fungal Research Trust

