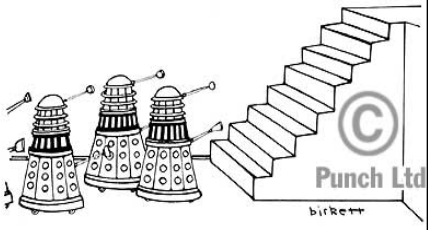
Impacts of Diagnostics on Antifungal Treatment

Have New Developments brought us any further ?

Importance of accurate diagnosis

- Mortality reduced by early treatment
- Allows better targeting of antifungal drugs
  - Reduction in empirical amphotericin
  - Reduced drug toxicities and costs
  - Optimal treatment for fungal pathogen
- Decreases delays in completion of chemotherapy
- Permits appropriate selection of secondary prophylaxis for future treatments or transplantation

Nosari et al Am J Hematol 2001;68:231-236



"Well, this certainly buggers our plan to conquer the Universe."

Consensus criteria

- Aimed to provide definitions for proven , probable and possible fungal infection that could facilitate **clinical research**
- Combined host, clinical and microbiological factors
- Not intended as **a guide to clinical practice**
- Focused on oncology and stem cell transplant populations

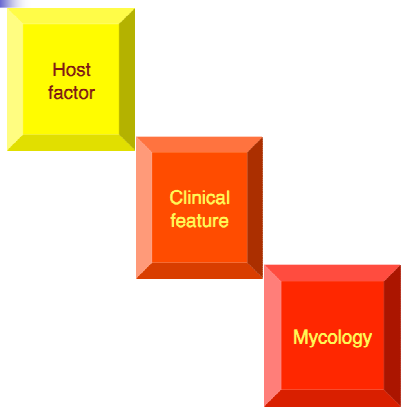
❖**Defining Opportunistic Invasive Fungal Infections in Immunocompromised Patients: An International Consensus Ascioglu et al CID 2002 34:7-114**
❖**Revised definitions of Invasive Fungal Disease de Pauw et al CID 2008:46**

Clinical trials

- Highly selective population
- Not representative of real life clinical practice
- Should not use the same criteria to develop care pathways for initiation of antifungal therapy

Defining invasive fungal disease

EORTC/BAMSG Consensus Revised definitions



- Host factors
  - Neutropenia
  - Allotransplantation
  - Prolonged steriods
  - other immunosuppressants and BRMs
  - Inherited severe immunodeficiency

Clinical factors:

Lower respiratory tract fungal disease

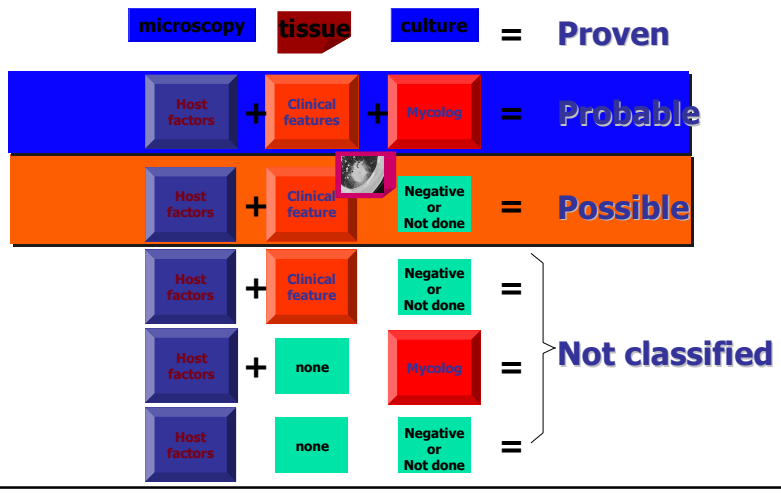
- presence of one of the following **"specific"** imaging signs on CT:-
  - Well defined nodule(s) with or without a halo sign
  - Wedge-shaped infiltrate
  - Air crescent sign
  - Cavity
- presence of a new non-specific focal infiltrate PLUS at least one of the following*
  - Pleural rub
  - Pleural pain
  - Hemoptysis

*symptoms not necessary if there is mycological evidence

Microbiological Criteria

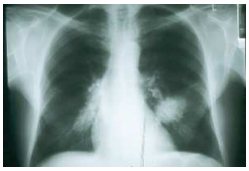
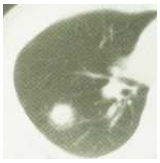
- Cytology, direct microscopy or culture
  - Sinus, sputum, BAL etc
  - Skin (microscopy and culture required)
- Detection of antigen, cell wall marker
  - single plasma, serum, BAL, pleural fluid or CSF sample positive for galactomannan
  - single serum sample positive for ß-D-glucan
  - PCR and nucleic acid methods NOT included

Invasive fungal disease - Definitions II

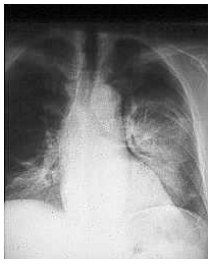


Halo sign

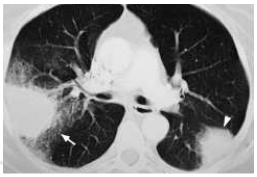
- What is it ?
- Means different things to different people
- Is it
  - displacement/necrosis/cavitation of lung tissue (no lung markings visible within halo)
  - infiltration/invasion of adjacent lung tissue (ground glass appearance)
- Both radiogically accepted definition
- Need to understand the pathogenesis

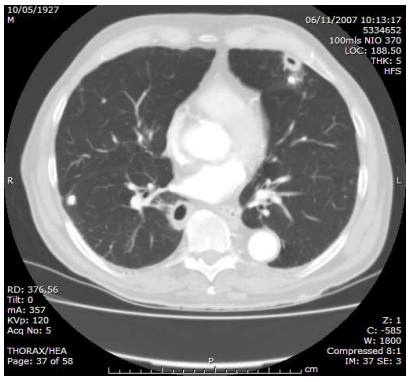

- displacement/necrosis/cavitation of lung tissue (no lung markings visible within halo)
  - Classically described in aspergillosis
    - Hruban et al: Radiologic-pathologic correlation of the CT halo sign in invasive pulmonary aspergillosis. J Comput Assist Tomogr 11: 534-536, 1987
- Angioinvasion by hyphae
  - lead to infarction of tissues
    - mycotic lung sequestrum
    - Wedge shaped infarcts
    - Necrotic tissues cavitates
      - With time
      - Neutrophil recovery



But since late 90s



- Nodule surrounded by ground glass appearance
  - due to infiltration/invasion of adjacent lung tissue
- Now appears to be uniformly accepted as virtually pathognomonic for IFI
- Very nonspecific
  - Other fungi
    - Aspergillosis, fusariosis, zygomycosis, candidosis, coccidiodomycosis
  - Other infection
    - TB, nocardia, organizing pneumonia, septic emboli
  - Malignancy
    - angiosarcoma, choriocarcinoma, osteosarcoma, Kaposi's
  - Vasculitides, eosinophilic lung disease



MAJOR ARTICLE

Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group

Ben De Pauw,* Thomas J. Walsh,* J. Peter Donnelly,* David A. Stevens, John E. Edwards, Thierry Calandra, Peter G. Pappas, Johan Maertens, Olivier Lortholary, Carol A. Kauffman, David W. Denning, Thomas F. Patterson, Georg Maschmeyer, Jacques Bille, William E. Dismukes, Raoul Herbrecht, William W. Hope, Christopher C. Kibbler, Bart Jan Kullberg, Kieren A. Marr, Patricia Muñoz, Frank C. Odds, John R. Perfect, Angela Restrepo, Markus Ruhnke, Brahm H. Segal, Jack D. Sobel, Tania C. Sorrell, Claudio Viscoli, John R. Wingard, Theoklis Zaoutis, and John E. Bennett*

Background. Invasive fungal diseases are important causes of morbidity and mortality. Clarity and uniformity in defining these infections are important factors in improving the quality of clinical studies. A standard set of definitions strengthens the consistency and reproducibility of such studies.

Methods. After the introduction of the original European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group definitions, advances in diagnostic technology and the recognition of areas in need of improvement led to a revision of this document. The revision process started with a meeting of participants in 2003, to decide on the process and to draft the proposal. This was followed by several rounds of consultation until a final draft was approved in 2005. This was made available for 6 months to allow public comment, and then the manuscript was prepared and approved.

Results. The revised definitions retain the original classifications of "proven," "probable," and "possible" invasive fungal disease, but the definition of "probable" has been expanded, whereas the scope of the category "possible" has been diminished. The category of proven invasive fungal disease can apply to any patient, regardless of whether the patient is immunocompromised, whereas the probable and possible categories are proposed for immunocompromised patients only.

Conclusions. These revised definitions of invasive fungal disease are intended to advance clinical and epidemiological research and may serve as a useful model for defining other infections in high-risk patients.

In 2002, a consensus group of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) published standard definitions for invasive fungal infections for clinical and epidemiological research [1]. These definitions were developed to facilitate the identification of reasonably homogeneous groups of patients for clinical and epidemiologic research, to help design clinical trials to evaluate new drugs and management strategies, and, last but not least, to foster communication between

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* B.d.P. and T.J.W. served as cochairs and J.P.D. served as secretary for the EORTC/MSG Consensus Group.

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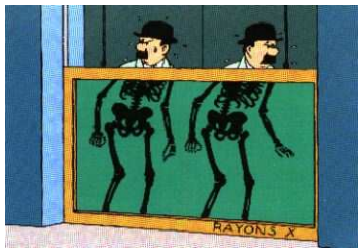
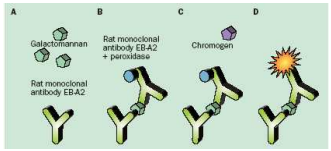
1058-4838/2008/4612-0003$15.00
DOI: 10.1086/588660

Radiological interpretation

- No radiological consensus
- still upgraded in new consnesus definitions
- Mycology downgraded



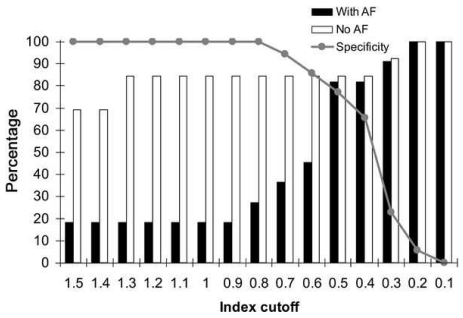
Biomarkers



- Galactomannan
- Other biomarkers
  - Beta-glucan
  - PCR
- Secondary metabolites
  - D-arabinitol/mannitol:L-arabinitol/mannitol ratios
  - positron emission tomography
  - Proteomic/metabolomics
- Have diagnostic utility
  - Provided limitations and interpretation understood
  - Less subjective
  - More cost effective
  - More rapidly available

Factors affecting performance

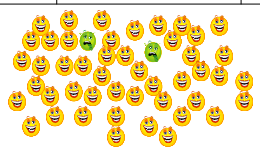
- EIA (Platelia)
  - OD cut-off used
  - HSCT >> SOT
  - Neutropenia vs non neutropenia
    - Different pathogenesis
    - Low fungal load
    - More immunopathology/cellular trafficking
    - Limits utility of biomarkers and CT scan
  - Prevalence of disease



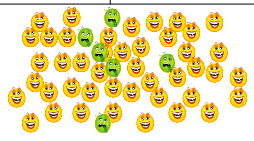
Marr KA et al J Infect Dis **2004**; 190:641–9

Pfeiffer et al. (2006) Diagnosis of Aspergillosis. Clin Infect Dis 42:1417-1727

	Cases of proven IA		Cases of proven or probable IA	
Prevalence	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
0.05	0.25 (0.23–0.28)	0.98 (0.97–0.99)	0.31 (0.28–0.35)	0.98 (0.97–0.99)
0.10	0.42 (0.39–0.45)	0.96 (0.95–0.97)	0.49 (0.45–0.53)	0.96 (0.95–0.97)
0.15	0.53 (0.50–0.56)	0.95 (0.94–0.96)	0.61 (0.57–0.64)	0.93 (0.92–0.94)
0.20	0.62 (0.59–0.65)	0.92 (0.91–0.94)	0.69 (0.65–0.72)	0.91 (0.89–0.92)



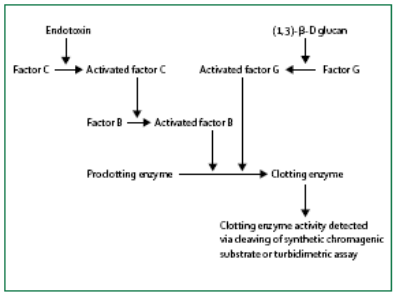
Prevalence: 5%
PPV 25%



Prevalence: 20%
PPV 67%

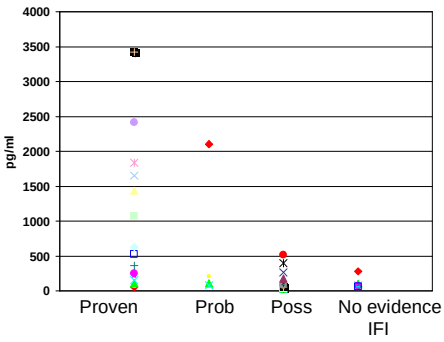
β-D-Glucan

- component of the cell wall
- activates factor G of the horseshoe crab coagulation cascade
- Detects down to 1 pg/ml
- Cannot distinguish different fungal species
- Species differ in amount of beta D glucan content in cell wall
  - Cryptococcus , 6%
  - Mucor, Rhizopus species <10%
  - Aspergillus and Candida: major cell wall constituent
- commercial assays available
  - Fungitec-G (Seikagaku) : cut off 20pg/ml
  - Glucatell (Associates of Cape Cod) : cut off 60pg/ml
  - expensive

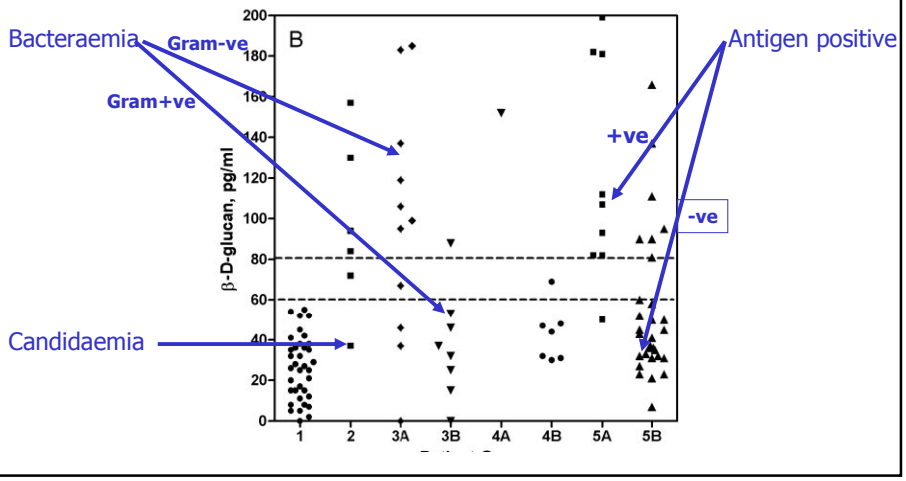


Odabasi et al. CID 2004;39:199-205

No. of BG-positive sera	Proven or probable IFI			
	Sens %	Spec %	PPV %	NPV, %
1 specimen	100	90	43	100
2 sequential specimens	65	96	57	97
3 sequential specimens	60	99	80	96



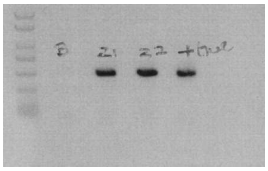
Pickering et al J Clin Microbiol 2005;43: 5957-62



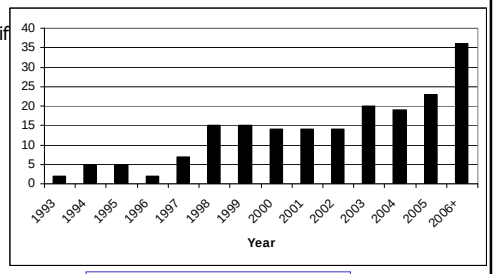
Molecular diagnosis

- Ideal
  - Should be sensitive (present early into the course of the disease)
  - But should not be too transient
  - capable of detecting non-culturable/viable cells or free DNA
  - Rapid turnaround time required
  - Low risk of contamination or colonisation
- Used to determine
  - Initiation of antifungal therapy (no more empiric therapy)
  - optimal duration of therapy
  - High negative predictive value is essential

Molecular diagnosis

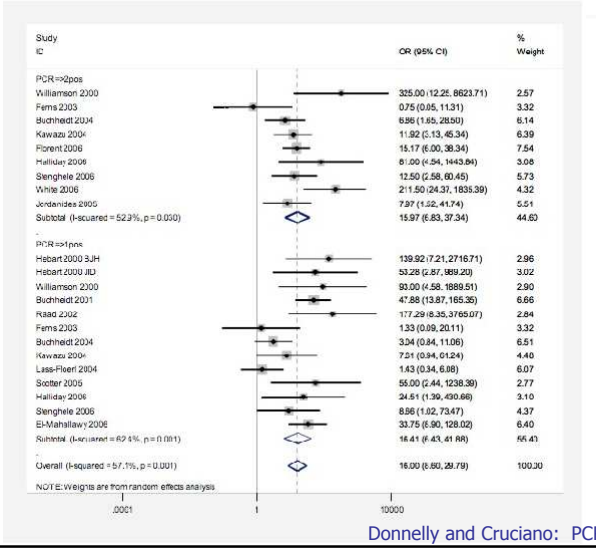


- Lengthy extraction procedure
- In house assays, lack of standardisation
- limited consensus on;
  - Specimen type
  - Extraction
  - target
    - panfungal or species specif
- contamination
- detection of product
  - real-time
  - probes
  - sequence
- cost



published articles

Forest plot showing diagnostic Odds ratios



Donnelly and Cruciano: PCR meta-analysis

The UK Fungal PCR consensus group

- In 2004, *Aspergillus* DNA, from known CFU values and PCR reagents for both assays were distributed.
  - 11 centres participated
  - In total each assay was performed 21 fold
  - 3 different real-time PCR platforms were used

	Sensitivity	Specificity	PPV	NPV
Assay 1	85.7%	93.5%	92.9%	86.7%
Assay 2	76.5%	83.8%	82.5%	72.1%

White PL et al A consensus on fungal PCR diagnosis? - A UK-Ireland evaluation of PCR methods for the detection of systemic fungal infections. J Mol Diagnostics 2006; 8: 376-384.

ISHAM
INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

WORKING GROUP
EUROPEAN ASPERGILLUS PCR INITIATIVE
EAPCRI

- **EAPCR – Laboratory Working Group**
- 24 centres
- PCR amplification methods are very consistent in their performance
  - 95% of methods detected the predicted 100% threshold
- 10 PCR methods were able to detect below threshold
  - Further evaluation is required
- Wide variation in the performance of extraction methods
  - Use of larger volumes of blood correlated with better performance
    - At least 4ml should be used
  - Bead-beating methods performed optimally when testing QC panel
    - Performance in clinical specimens
    - To be evaluated in clinical trial

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INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

WORKING GROUP
EUROPEAN ASPERGILLUS PCR INITIATIVE
EAPCRI

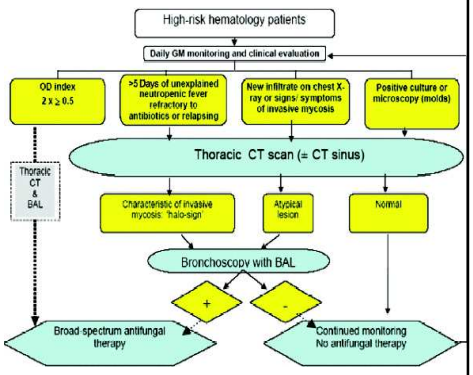
- PCR interpretation
  - Close to a European standard methodology
  - Need to consider how we use these tests
  - Not necessarily to diagnose IFI
    - PPV similarly affected by prevalence of disease but NPV remains high
  - But as a screen to rule out IFI
    - Empirical treatment (and prophylaxis in areas of low prevalence) become unnecessary

Can biomarkers be used for diagnosis

- PCR assays and immunoassays (GM EIA) have been studied
  - Particularly strong negative predictive values
- Can diagnostic assays be used to limit empiric therapy
- Is this safe

Galactomannan EIA

- 136 episodes of neutropenia
  - Patients receiving flucon prophylaxis
- daily EIA GM + early CT scanning in neutropenic febrile episodes
- Antifungal given if 2 consecutive EIA GM results +ve and confirmed by BAL or CT
  - 3 breakthrough infections
    - 2 candidemias
    - 1 mucorales
  - No excess mortality or fungal related death
  - No impact on overall antifungal usage despited deceased empirical use



Maertens et al. Clin Infect Dis 2005; 41: 1242

Galactomannan EIA

- 293 patients haem malignancies randomised
- empirical or pre-emptive therapy
- Patients were screened for GM
- empirical arm received antifungals if they had persistent fever
- pre-emptive patients given antifungal only if they showed clinical signs or had a positive GM
- Survival was not significantly
- pre-emptive patients received significantly less antifungals
- no significant cost savings were achieved

Cordonnier C *et al.* Blood 2006;108: 572A.

PCR

- Nested PCR to guide antifungal therapy
  - 42 patients with cancer, neutropenia
  - AmB required in only 2 patients

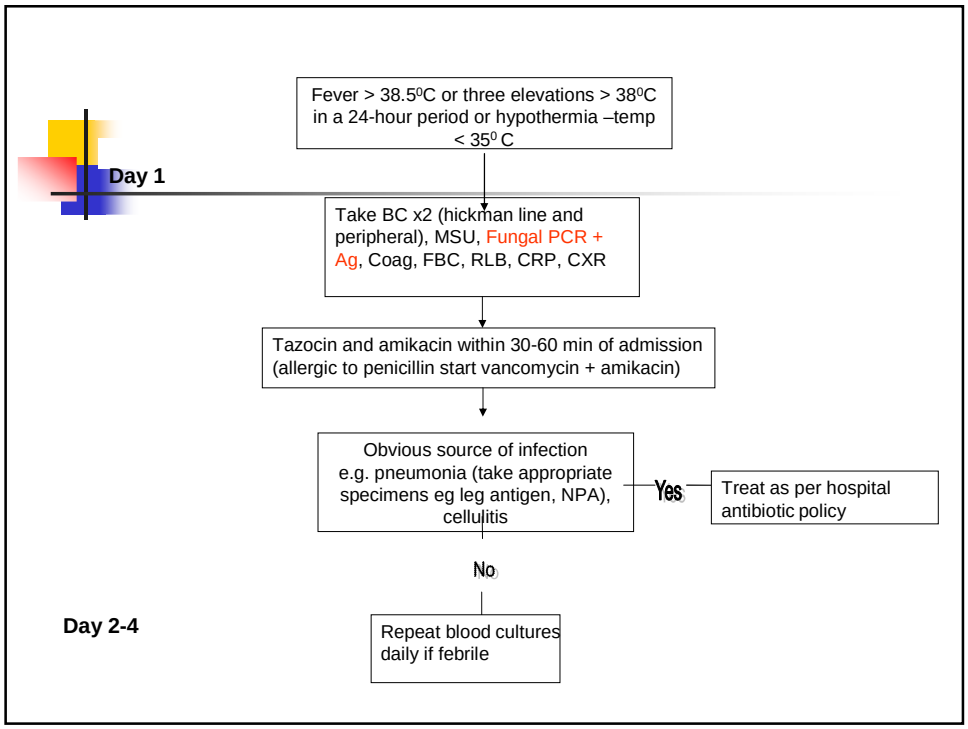
Lin et al. *Clin Infect Dis.* 2001;33:1621-1627

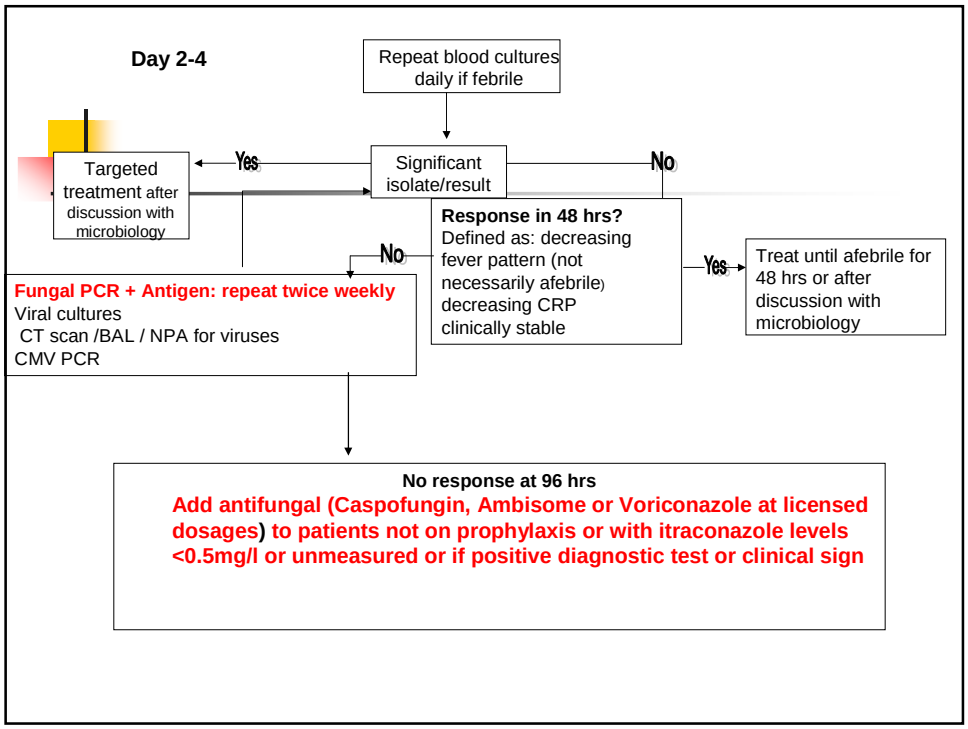
- randomised study of a PCR directed versus an empirical antifungal
  - more than 400 SCT patients
  - safe
  - No reduction in antifungal drug use.

Hebart *et al.* Blood 2004;104: 59A.

Cardiff experience

- 125 patients with febrile neutropenia on single unit
- haematological malignancy
- Undergoing SCT or remission induction chemotherapy
- High risk patients received Itraconazole solution prophylaxis (with weekly levels) or AmBisome 7mg/kg weekly
- Twice weekly aspergillus and candida PCR and ELISA during neutropenic fever or GVHD
- According to new care pathway

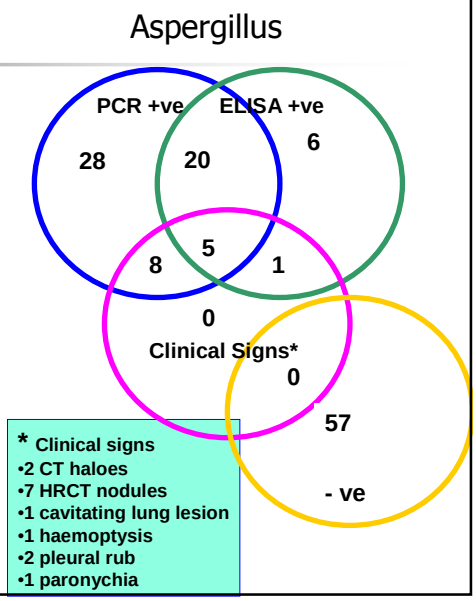

Day 1
Fever > 38.5⁰C or three elevations > 38⁰C in a 24-hour period or hypothermia –temp < 35⁰ C
Take BC x2 (hickman line and peripheral), MSU, Fungal PCR + Ag, Coag, FBC, RLB, CRP, CXR
Tazocin and amikacin within 30-60 min of admission (allergic to penicillin start vancomycin + amikacin)
Obvious source of infection e.g. pneumonia (take appropriate specimens eg leg antigen, NPA), cellulitis
Yes
Treat as per hospital antibiotic policy
No
Day 2-4
Repeat blood cultures daily if febrile


Day 2-4
Repeat blood cultures daily if febrile
Significant isolate/result
Yes
Targeted treatment after discussion with microbiology
No
Response in 48 hrs?
Defined as: decreasing fever pattern (not necessarily afebrile) decreasing CRP clinically stable
Yes
Treat until afebrile for 48 hrs or after discussion with microbiology
No
Fungal PCR + Antigen: repeat twice weekly
Viral cultures
CT scan /BAL / NPA for viruses
CMV PCR
No response at 96 hrs
Add antifungal (Caspofungin, Ambisome or Voriconazole at licensed dosages) to patients not on prophylaxis or with itraconazole levels <0.5mg/l or unmeasured or if positive diagnostic test or clinical sign

Neutropenic care pathway

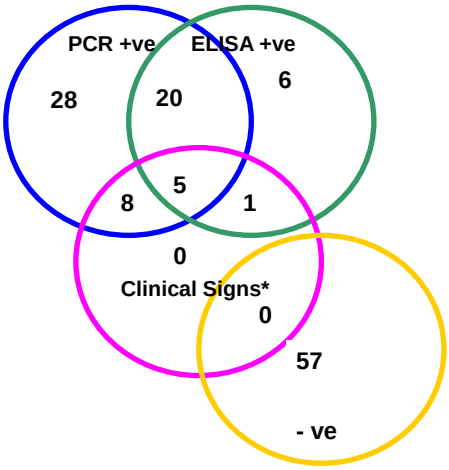
- Introduced Oct 2005 to incorporate molecular diagnostics
  - PCR and ELISA
- Empirical antifungal arm removed for:
  - Patients on effective prophylaxis
    - Itraconazole with serum levels >/= 0.5 mg/l
    - AmBisome 7mg/kg weekly
  - Unless directed by positive diagnostic test or clinical signs
- Audited 6 month cohort with one-year follow up

- 125 patients screened
  - 514 specimens tested by PCR
  - 501 By ELISA
- EORTC/MSG criteria used to classify IFI
- During the test period
  - 1 case confirmed invasive candidosis
- Prevalence 6%
  - (12% if PCR included)

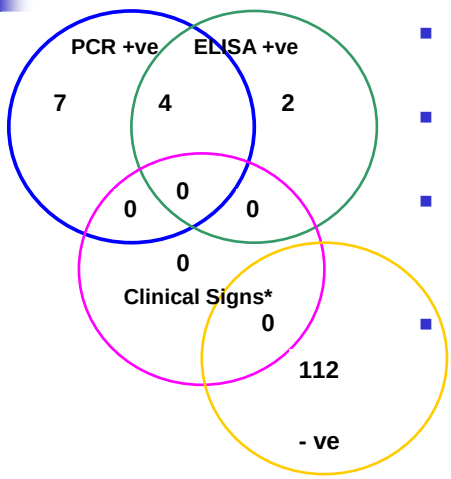


During 12 month follow up

- 2 possible patients had IFD confirmed
- two possible patients (PCR and EIA GM) moved into a probable category with clinical signs consistent with IFD
- One patient negative during test period developed fungal sinusitis and became PCR and antigen positive
- Prevalence 12%
  - (15% if PCR included)



Candida infection



- 11 patients positive by PCR
  - All but 2 also asp PCR +ve
- 6 patients (11 specimens) positive by ELISA
- 4 patients positive by both PCR and ELISA
  - 1 blood culture confirmed
- 10 patients colonised (skin, mucus membranes and urine)

		Crude Mortality (%)	Attributable mortality (%)	Fungal free survival (%)	Patients with ongoing IFD
Aspergillosis n=25	PCR + GM EIA positive	40·0	24.0	40.0	6
n=36	PCR positive	44·4	8·3	52·8	1
n=7	GM EIA	57·1	0	42·9	
Candida n=9	PCR + M EIA positive	77·8	0	22·2	
n=2	PCR positive	50	0	0 (0)	
n=1	M EIA	100	0	0	
Negative by all tests n= 55		23·6	0	76·4	
Total		33·6	8.0	60·8	5.6

Likelihood ratio

- Likelihood of a positive result in a patient with proven/probable disease versus positive result in a patient without evidence of disease
- Not affected by prevalence (unlike PPV)
- LR = sensitivity/1-specificity

	Single non reproducible positive PCR	Single reproducible positive PCR	Multiple PCR positive
Likelihood ratio	2.5	3.6	7.4*

* Rises to 9.6 if PCR were t be included in EORTC/MSG criteria

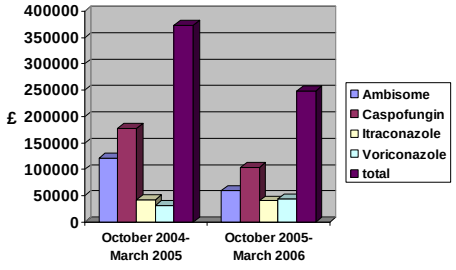
Negative group

- 55 patients
- Persistently negative by all tests
- 6 received empirical antifungal during refractory fever ("fear factor")
- No patients had clinical features to suggest IFI
  - 4 had evidence of candida colonisation
  - 10 had CT thorax performed
    - None High res, 2 CTPA

Costs

- Full economic costing (FEC)
  - consumables, labour VAT, 15% wastage and 30% overheads
- Antifungal drug expenditure fell by £124,570 (€ 173,055)

Costs	FEC	
	£	€
Antigen	48.60	67.50
PCR	31.80	44.20
Total for our unit annually	143470	199420



Other impacts

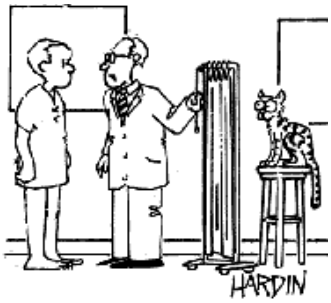
- Bed occupancy not significantly different
  - towards decreased length of stay (average 6.6 days compared to 7.2 days)
  - finished consultant episodes increased slightly (376 compared to 366)
- Other clinical outcomes yet to be assessed
  - Decreased drug associated adverse events/morbidities
    - nephrotoxicity
  - delays in underlying remission-induction treatment

Conclusions

- Implementation of molecular diagnostics enables a move away from empirical therapy to targeted pre-emptive therapy
- Costs of implementation can be easily met by decreased antifungal drug usage
- Further beneficial impacts from earlier diagnosis and reduction of adverse drug events associated with empirical therapies likely to be realised

Summary of diagnosis

- Diagnosis requires a multidisciplinary approach
  - Clinical
  - Microbiological
  - histological
  - Radiological
- Use all available information



"Step up to the curtain and we'll begin the cat scan.