Research priorities in medical mycology

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Agenda

How many patients are there with serious fungal infection?
Acute versus recurrent versus chronic infection
Diagnostics – progress and gaps
Risk evaluation using genetics – how likely?
Prophylaxis versus vaccines
Antifungal resistance and new antifungals
Funding issues
Conclusions

The size of the problem

Over 300 million people affected by serious Fungal Infection worldwide

www.fungalresearchtrust.org/HowCommonareFungalDiseases2.pdf

LIFE
LEADING INTERNATIONAL FUNGAL EDUCATION

The size of the problem

Fungal Infection	Global burden of serious fungal infection (estimates by underlying disease)				
	None	HIV/AIDS	Respiratory	Immune deficit / Cancer	Critical care
Cryptococcal meningitis					
Pneumocystis pneumonia					
Invasive aspergillosis					
Chronic pulmonary aspergillosis					
Fungal eye infection					
Fungal hair infection					

The size of the problem

Fungal Infection	Global burden of serious fungal infection (estimates by underlying disease)				
	None	HIV/AIDS	Respiratory	Immune deficit / Cancer	Critical care
Cryptococcal meningitis	1,000's	1,000,000		1,000's	
Pneumocystis pneumonia		>200,000		>100,000	
Invasive aspergillosis			>100,000	>50,000	>50,000
Chronic pulmonary aspergillosis			3,000,000		
Fungal eye infection	1,000,000				
Fungal hair infection	200 million				

The size of the problem

Fungal Infection	Global burden of serious fungal infection (estimates by underlying disease)				
	None	HIV/AIDS	Respiratory	Immune deficit / Cancer	Critical care
Candida infections					
Oral thrush					
Oesophageal candidasis					
Candida vaginitis 4x/yr	>75 million				
Candida bloodstream infection					

The size of the problem

Fungal Infection	Global burden of serious fungal infection (estimates by underlying disease)				
	None	HIV/AIDS	Respiratory	Immune deficit / Cancer	Critical care
Candida infections					
Oral thrush		9,500,000	100,000's	millions	
Oesophageal candidasis		2,000,000			
Candida vaginitis 4x/yr	>75 million				
Candida bloodstream infection				100,000	200,000
Allergic lung disease					
ABPA			4,000,000		
SAFS			>3,500,000		

The severity of the problem

Deaths per year

- Cryptococcal meningitis – 10% death rate in the USA, >80% in Africa. 600,000 deaths.
- Invasive aspergillosis – 50% mortality treated, 100% if not. >100,000 deaths
- Chronic pulmonary aspergillosis – 15% annual mortality, 450,000 deaths.
- Pneumocystis pneumonia - ~15% mortality in AIDS, ~50% non-AIDS, >80,000 deaths.
- Candida bloodstream infection - ~40% mortality, 120,000 deaths
- SAFS – increased risk of asthmatic death (estimated to be 100,000 annually worldwide)

Reality check with TB

	TB (2008)	Fungal Infection
Incident cases	**9-10 million**	**>14 million**
Prevalent cases	**10-13 million**	**~285 million**
HIV related deaths	**~550,000**	**~650,000**
Non-HIV related deaths	**~1,500,000**	**>700,000**

Chronic fungal infections

Fungal Infection	Global burden of serious fungal infection (estimates by underlying disease)				
	None	HIV/AIDS	Respiratory	Immune deficit / Cancer	Critical care
Cryptococcal meningitis	1,000's	1,000,000		1,000's	
Pneumocystis pneumonia		>200,000		>100,000	
Invasive aspergillosis			>100,000	>50,000	>50,000
Chronic pulmonary aspergillosis			3,000,000		
Fungal eye infection	1,000,000				
Fungal hair infection	200 million				

Recurrent and chronic fungal infections

Fungal Infection	Global burden of serious fungal infection (estimates by underlying disease)				
	None	HIV/AIDS	Respiratory	Immune deficit / Cancer	Critical care
Candida infections					
Oral thrush		9,500,000	100,000's	millions	
Oesophageal candidasis		2,000,000			
Candida vaginitis 4x/yr	>75 million				
Candida bloodstream infection				100,000	200,000
Allergic lung disease					
ABPA			4,000,000		
SAFS			>3,500,000		

The severity of the problem

Ill health and morbidity

- Oral and oesophageal thrush – unpleasant, reduced food intake and weight loss.
- Candida vaginitis – anxiety and impaired sex life
- ABPA and SAFS – breathlessness with severe asthma, reducing work capability
- Chronic pulmonary aspergillosis – progressive breathlessness and weight loss
- Fungal eye infection – unilateral blindness
- Fungal hair infection – psychological problems and contagious

Fungal Infection Impact

No studies assessing:

Disability Adjusted Life Years (DALY)
Quality Adjusted Life Years (QALY)
Quality-adjusted life expectancy (QALE)
Population health-related quality of life (HRQOL)

Diagnostic improvements in fungal diagnosis in last 20 years

- Aspergillus antigen testing
- Susceptibility testing of Candida and Aspergillus
- Chromagar
- CT scanning of the chest
- PCR for Pneumocystis, Aspergillus, Candida and Trichophyton
- Molecular identification of fungi and discovery of numerous cryptic species
- Direct identification from blood culture or agar plates
- Rapid dip-stick test for cryptococcal meningitis

Limitations of current diagnostics

a) insensitive
b) slow

Rapid diagnostic approaches

	Candida	Aspergillus	Mucorales	PCP
CRP	+/-	+/-	-	-
CT scan	+/-	++	+	-
Microscopy	+/-	+	++	++/+
GM antigen	-	++	-	-
Glucan	++	+	-	++
Antibody	+/-	+/-	-	-
PCR	+++	++	?+	+++

Candida blood cultures – performance of lysis centrifugation system

Autopsy diagnosis	Proportion B/C +ve (%)	Number of B/C drawn (median per pt)	Time to +ve (mean days)
Single organ	5/18 (28%)	11 (1-40)	3.2 (2-5)
Disseminated	11/19 (58%)	17 (6-55)	2.6 (1-4)
All	16/37 (43%)	-	-

Berenguer, Diagn Microbiol Infect Dis 1993;17:103.

Impact of fluconazole on *Candida* blood cultures in leukaemia

Sensitivity of blood culture for diagnosis of *Candida* species.

Year	Number of cases	*Candida albicans**	Other *Candida* spp.
1980–84	33	6/16	3/3
1985–89	32	1/13	3/7
1990–94	19	1/6	3/8
1995–99	10	0/2	2/6
Total	94	8/37*	11/24**

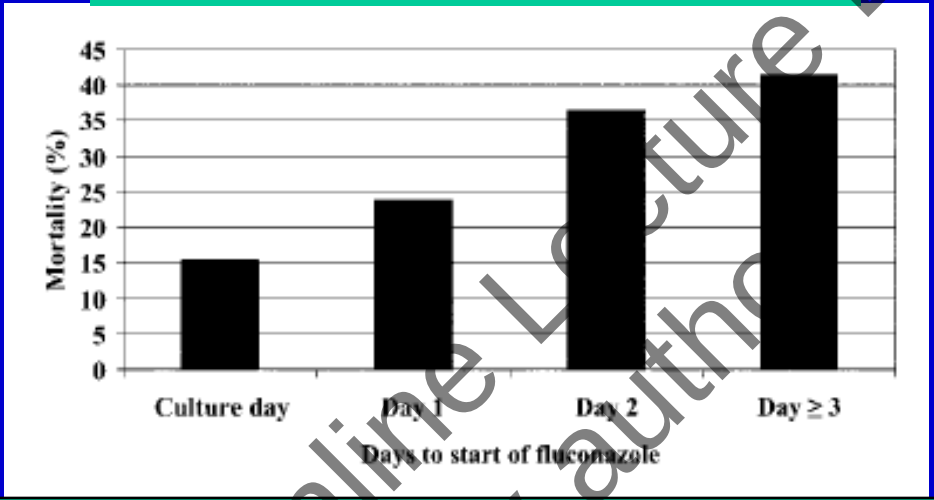
Autopsy proven cases of disseminated candidiasis – 20/94 (21%) with IC had a positive blood culture

Impact of fluconazole on negative cultures
P = 0.018 for all *Candida* species and
P = 0.0086 for *C. albicans*

Kami et al, Br J Haematol 2002;117:40.

Early treatment critical to good outcome

Candidaemia

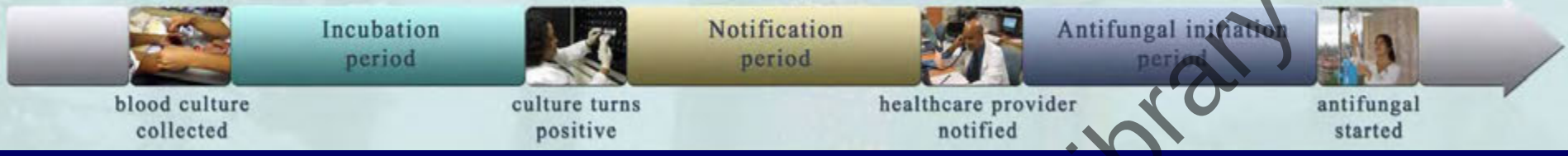


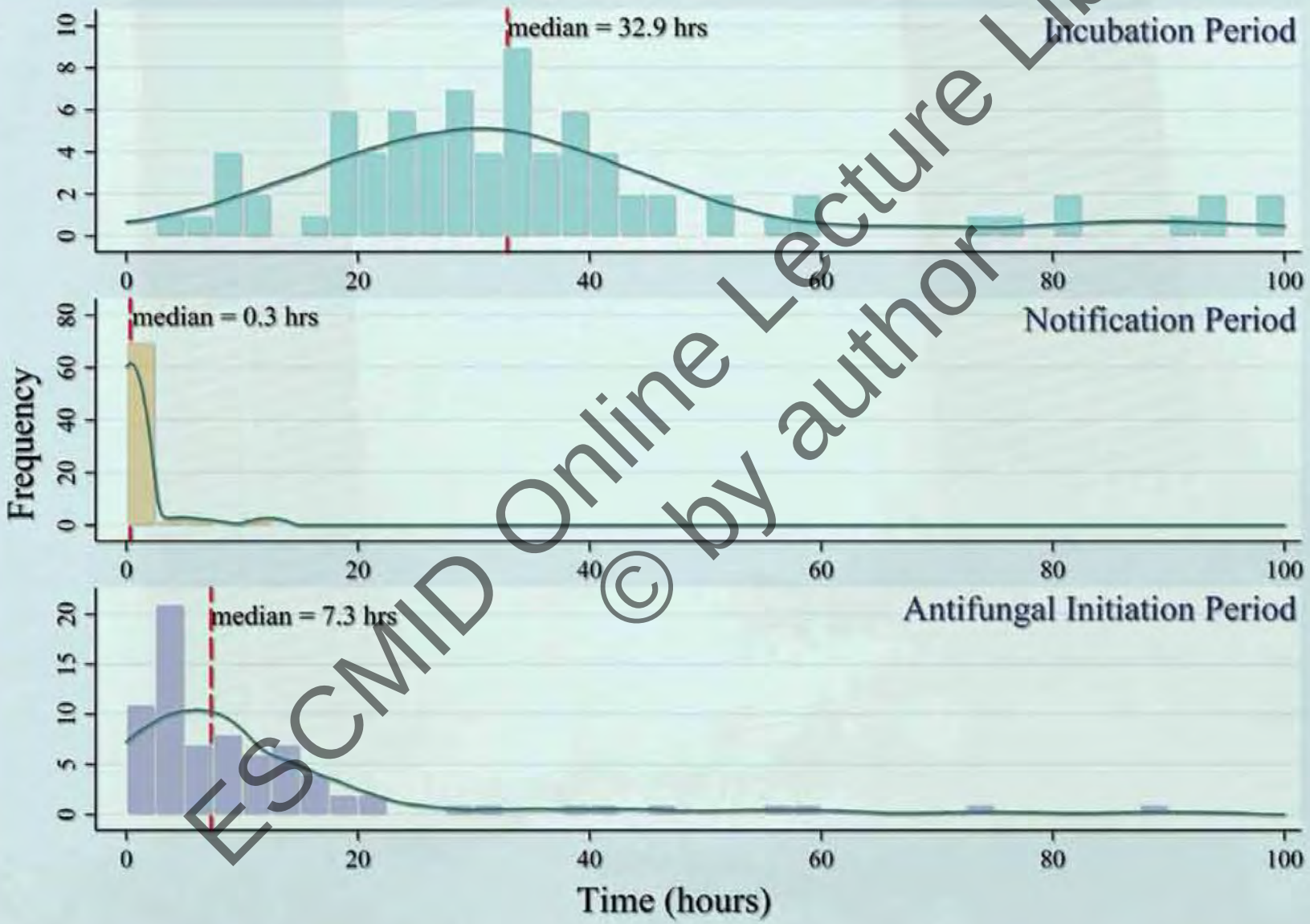
	Mortality rate from time of blood draw that later turns positive				
	Rx in <12 hrs	Rx in 12-24 hrs	Rx in 24-48 hrs	Rx >48 hrs	Rx >72 hrs
Morell, 2005	11.1%	30%	32.6%	34.5%	-
Garey, 2006		15.4%	23.7%	36.4%	41.4%

25%

25%

Morrell, Antimicrob Agents Chemother 2005;49:3640. Garey, Clin Infect Dis 2006;43:25





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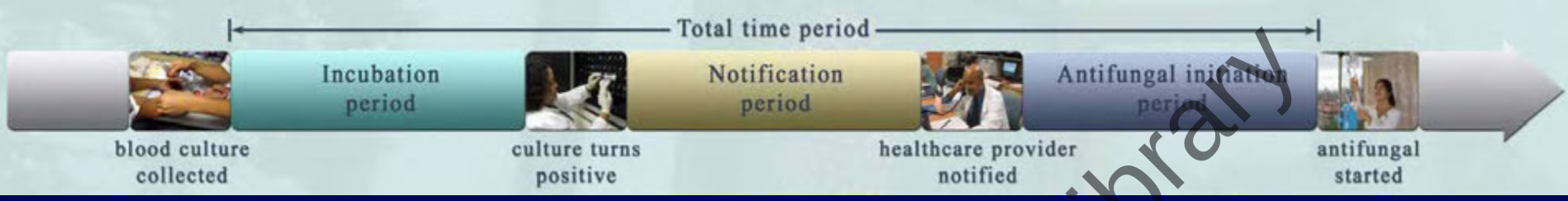


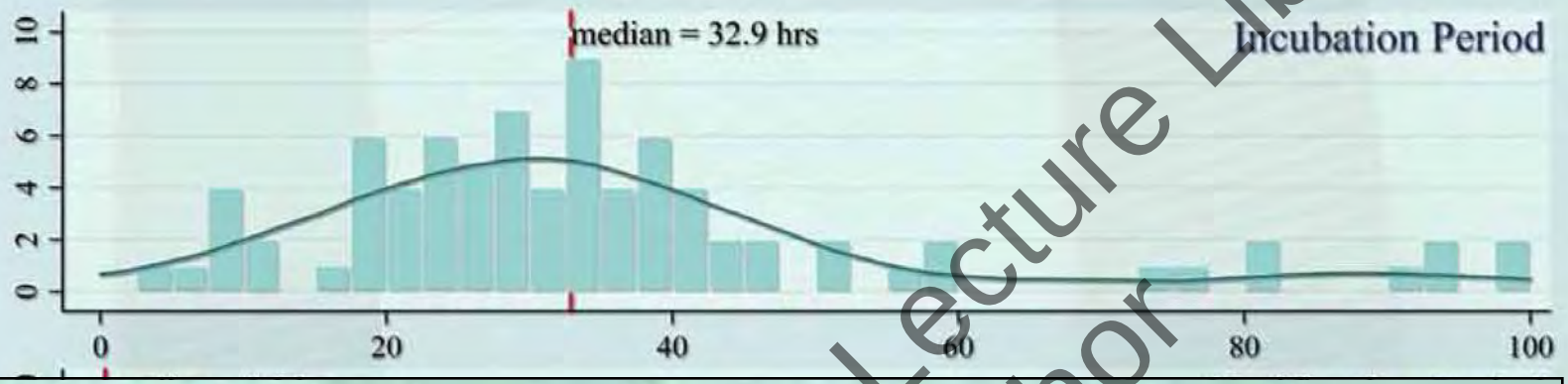
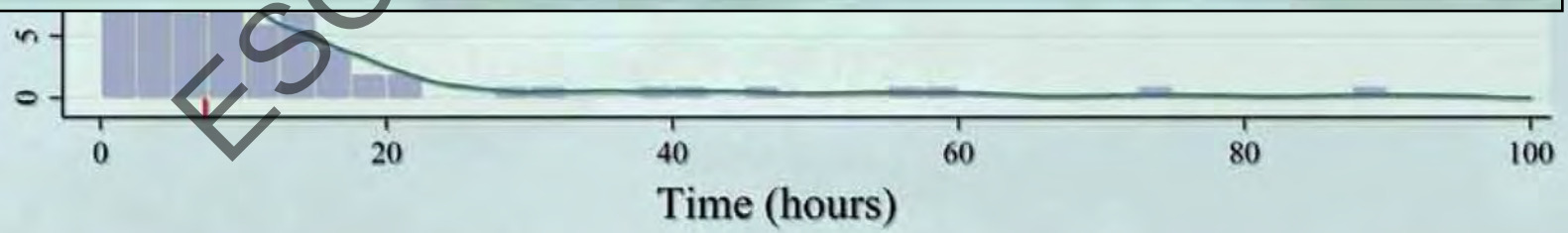


Table 3: Multivariate Predictors of Hospital Mortality

Variable	Multivariate Haz. Ratio	*P*-value	95% CI
Age (per year)	1.036	0.041	(1.001-1.073)
APACHE III (per point)	1.009	0.474	(0.984-1.035)
Incubation period (per hour)	1.021	0.021	(1.003-1.039)

This equates to an 2.4% increase in mortality per incubation hour



ICAAC 2007 K-2173

Meta-analysis of PCR for candidaemia and invasive candidiasis

PCR Diagnosis of Invasive Candidiasis: Systematic Review and Meta-Analysis▽†

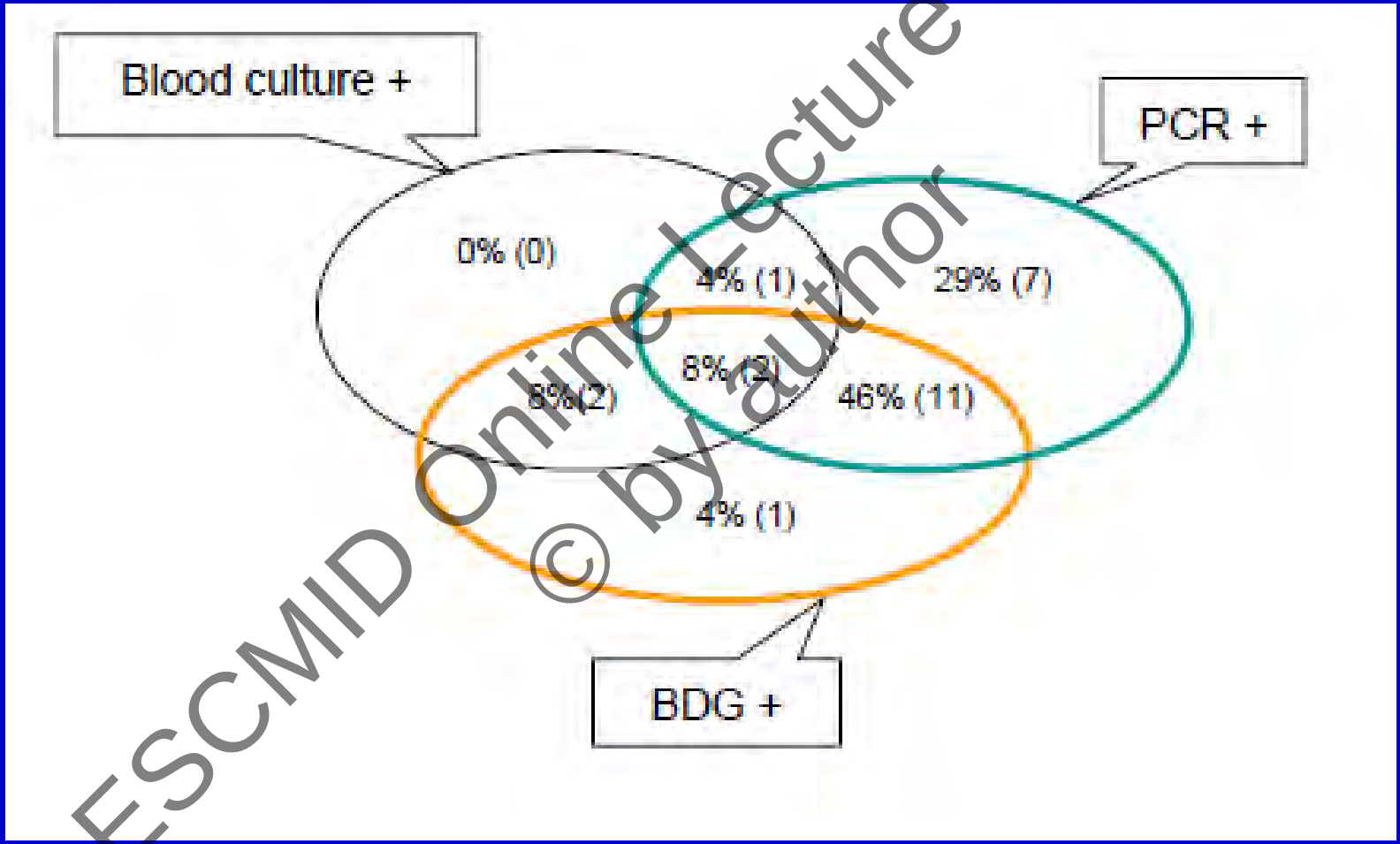
Tomer Avni,[1]* Leonard Leibovici,[1] and Mical Paul[2]

Medicine E[1] and Unit of Infectious Diseases,[2] Rabin Medical Center, Beilinson Hospital and Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel

or specificity values. We included 54 studies with 4,694 patients, 963 of whom had proven/probable or possible IC. Perfect (100%) sensitivity and specificity for PCR in whole-blood samples was observed when patients with cases had candidemia and controls were healthy people. When PCR was performed to evaluate patients with suspected invasive candidiasis, the pooled sensitivity for the diagnosis of candidemia was 0.95 (confidence interval, 0.88 to 0.98) and the pooled specificity was 0.93 (0.88 to 0.95). A specificity of >90% was maintained

targets and a PCR detection limit of ≤10 CFU/ml were associated with improved test performance. PCR positivity rates among patients with proven or probable IC were 85% (78 to 91%), while blood cultures were positive for 38% (29 to 46%). We conclude that direct PCR using blood samples had good sensitivity and

Diagnosis of candidaemia and invasive candidiasis with glucan and serum PCR



Direct detection of resistance mutations in clinical specimens, without positive cultures

Laboratory result	ABPA	CPA	Normals
Culture positive for *A. fumigatus*	0/19	7/42 (16.7%)	0/11
qPCR positive for *Aspergillus* spp	15/19 (78.9%)	30/42 (71.4%)	4/11 (36.4%)
A. fumigatus CYP51A mutation detected directly from qPCR positive sample	6/8 (75%)	12/24 (50%)	NT

Evaluation of processing methods for Aspergillus – sputa and bronchoscopy samples

Literature review

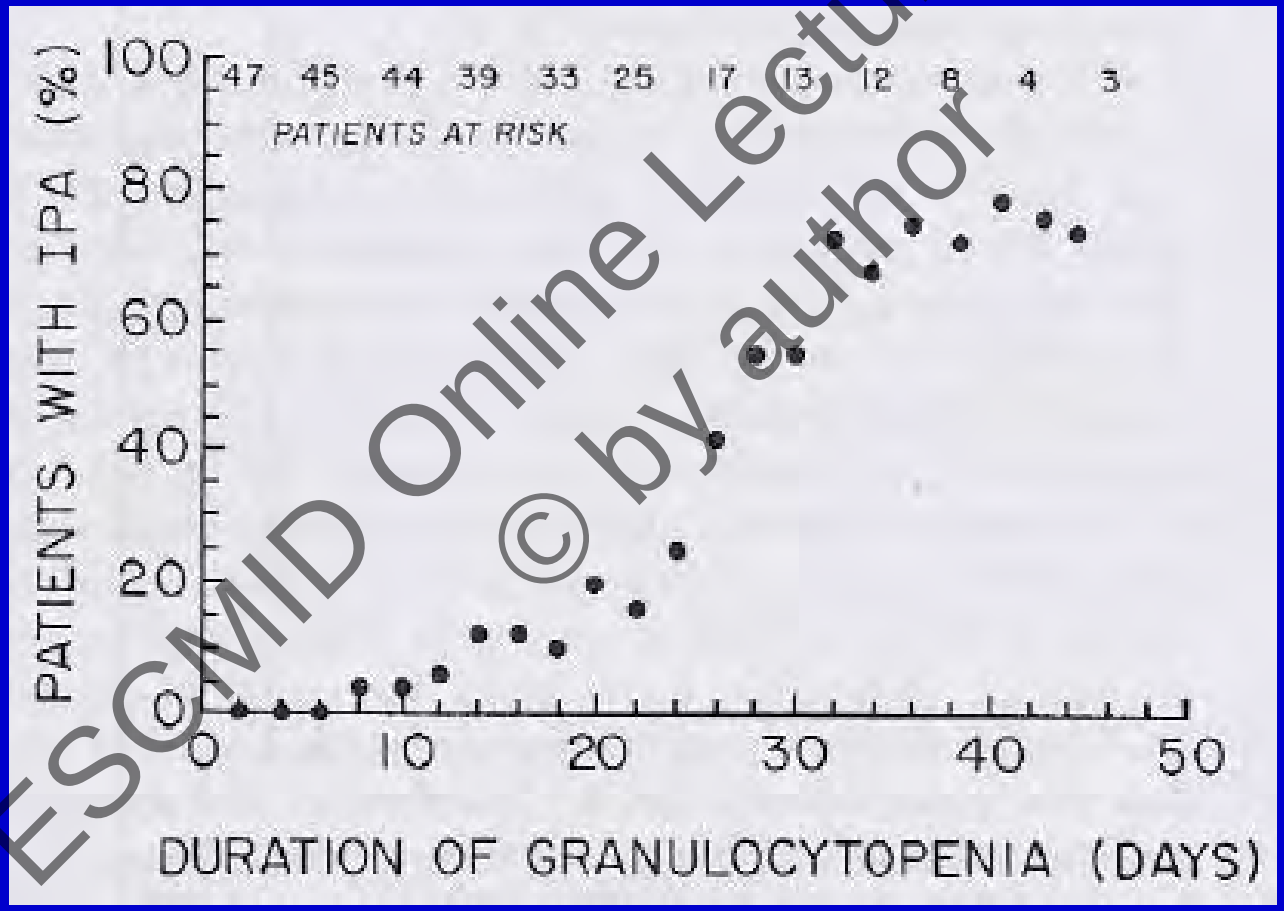
No papers

Invasive fungal disease risk assessment
Can we do it with genetics?

Invasive aspergillosis: Time of diagnosis

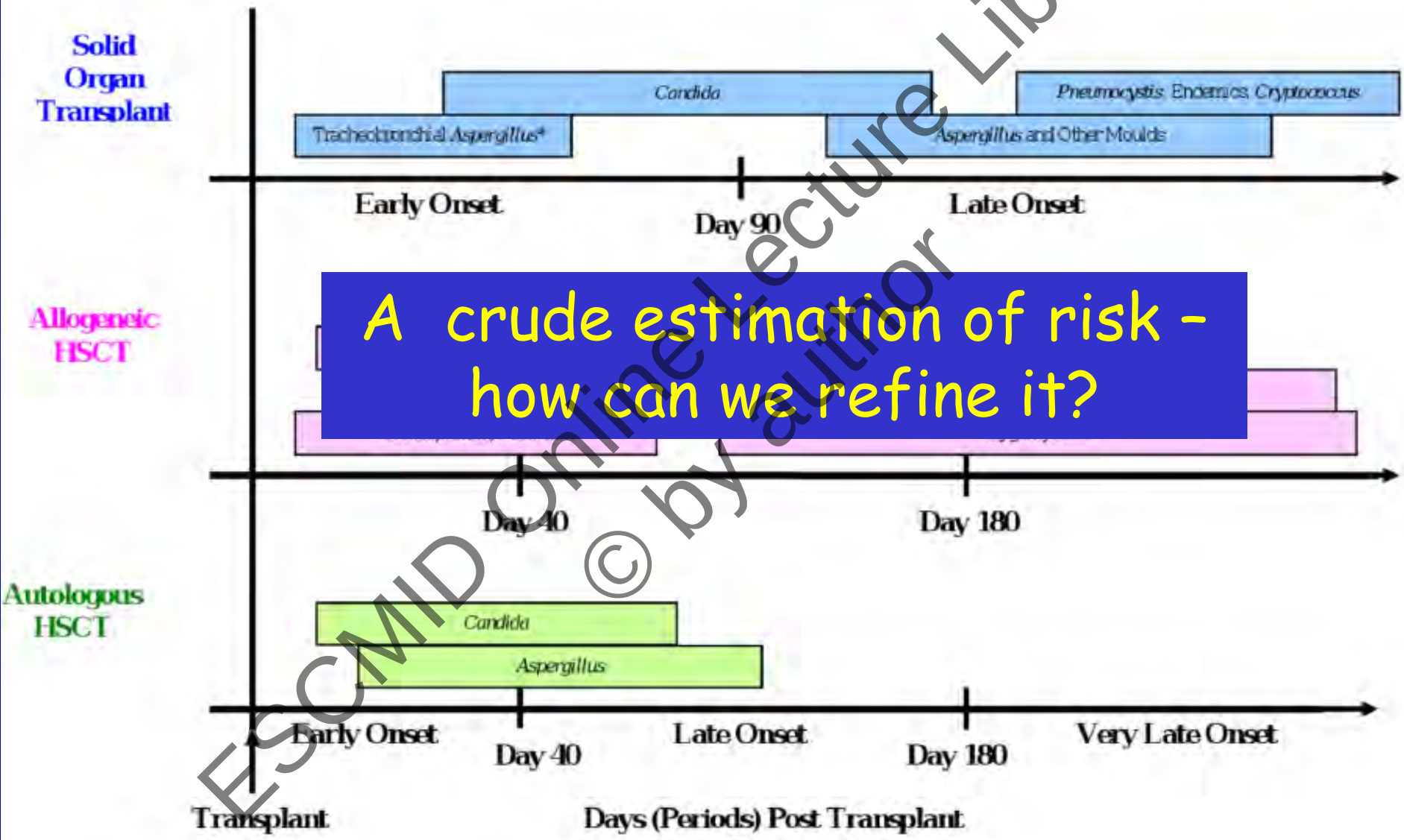
A single centre case control study :

- IA based on radiology (CXR) and clinical features



Gerson, Ann Intern Med 1984; 100:345

Risk period of fungal disease



A crude estimation of risk – how can we refine it?

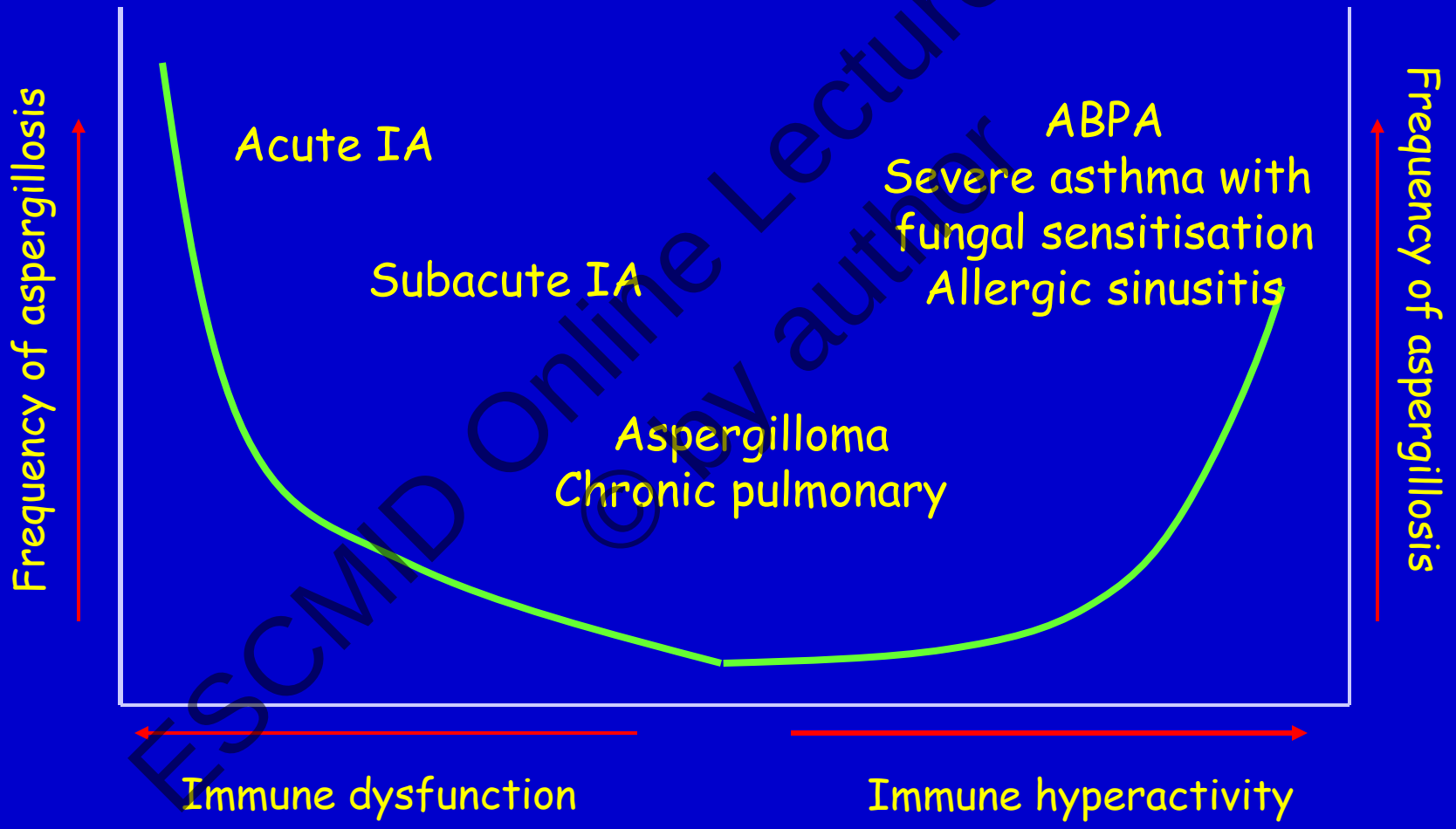
Pearson et al, Infect Dis Clinic N Am 2010;24:439

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	$p = 0{,}007$ OR = 2,2 CI = 1,2–3,8	Caucasian (retrospective)	IA after HSCT [EORTC/IFICG]	Mezger et al. (2008)
	rs3921	1642 C/G[a] [3′ UTR]	39	46	$p = 0{,}003$ OR = 2,6 CI = 1,4–5,0			
	rs4257674	−1101 A/G[a] [Promotor]	52	44	$p = 0{,}001$ OR = 2,8 CI = 1,6–5,2			
IFN-γ (12q14)	rs2069705	−1616 C/T[a] [Promotor]	69	56	$p = 0{,}010$ OR = 2,0 CI = 1,2–3,4			
	rs1800896	−1082 A/G [Promotor]	58	55	$p = 0{,}046$ OR = 1,7 CI = 1,0–2,9			
IL-10 (1q31-q32)	rs1878672	2068 C/G[a] [Intron]	67	57	$p = 0{,}025$ OR = 1,8 CI = 1,1–2,9			
	rs1800896	−1082 A/G [Promotor]	119 *Af* col. 27 ABPA	232	$p = 0{,}020$ OR = 1,7 CI = 1,1–2,5	Caucasian (retrospective)	colonization with *A. fumigatus* or ABPA after CF	Brouard et al. (2005)
	rs1800896 rs1800871 rs1800872 (haplotype)	−1082 A/G −819 C/T −592 A/C [Promotor]	9	96	* $p = 0{,}012$ OR = 9,3 CI = 1,6–52,8	Korean (retrospective)	IPA after HSCT [EORTC/MSG]	Seo et al. (2005)
	rs1800896	−1082 A/G [Promotor]	59	61	$p = 0{,}052$ OR = 1,7 CI = 1,0–2,9	Caucasian (prospective)	IPA in haematological patients [EORTC/IFICG]	Sainz et al. (2007b)
IL-1β (2q14)	rs1143627	−511 C/T [Promotor]	59	51	$p = 0{,}095$ OR = 1,7 CI = 0,9–3,0	Caucasian (retrospective)	IPA in haematological patients [EORTC/IFICG]	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)

Interaction of *Aspergillus* with the host
A unique microbial-host interaction



After Casadevall & Pirofski, Infect Immun 1999;67:3703

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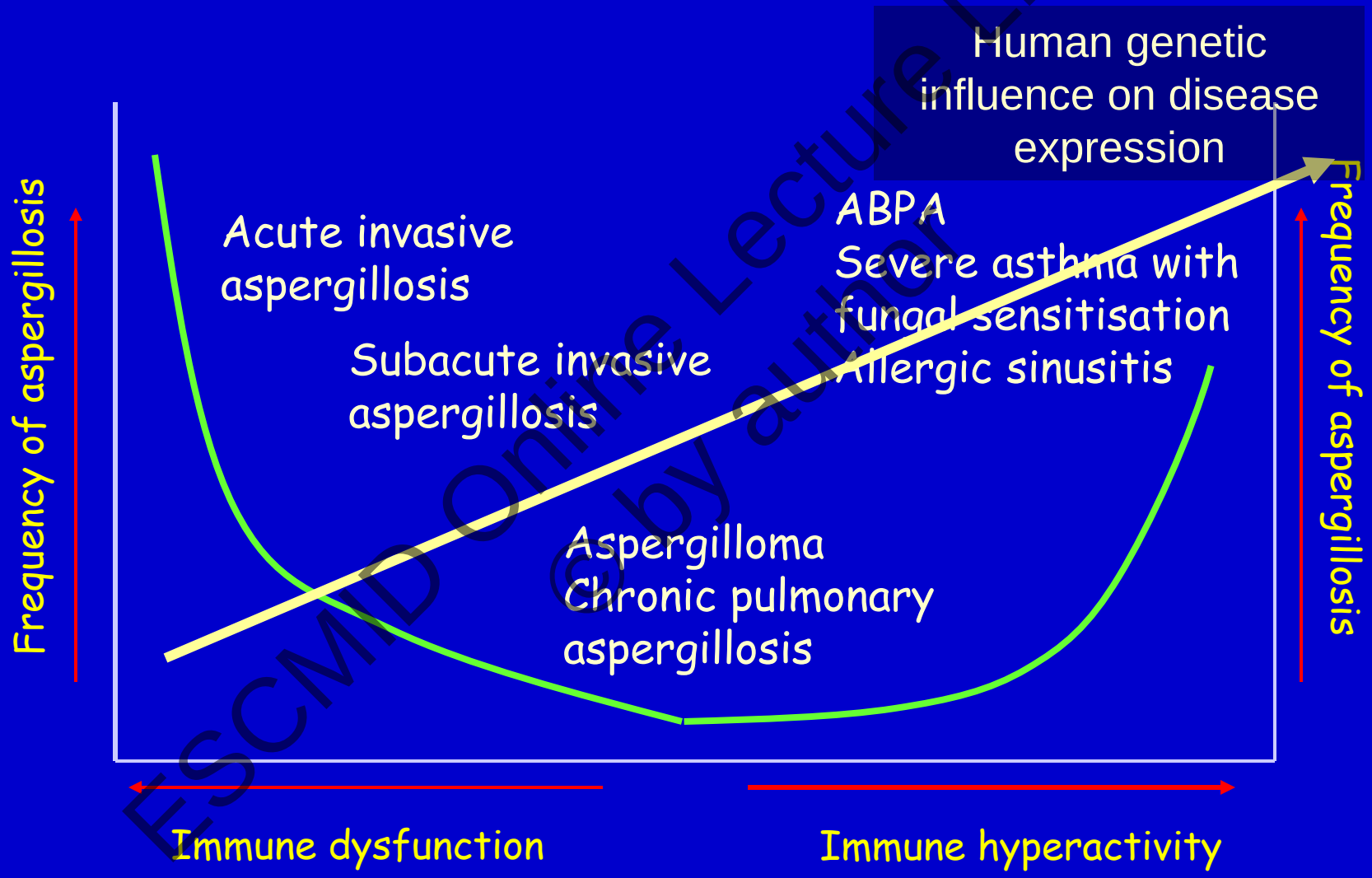


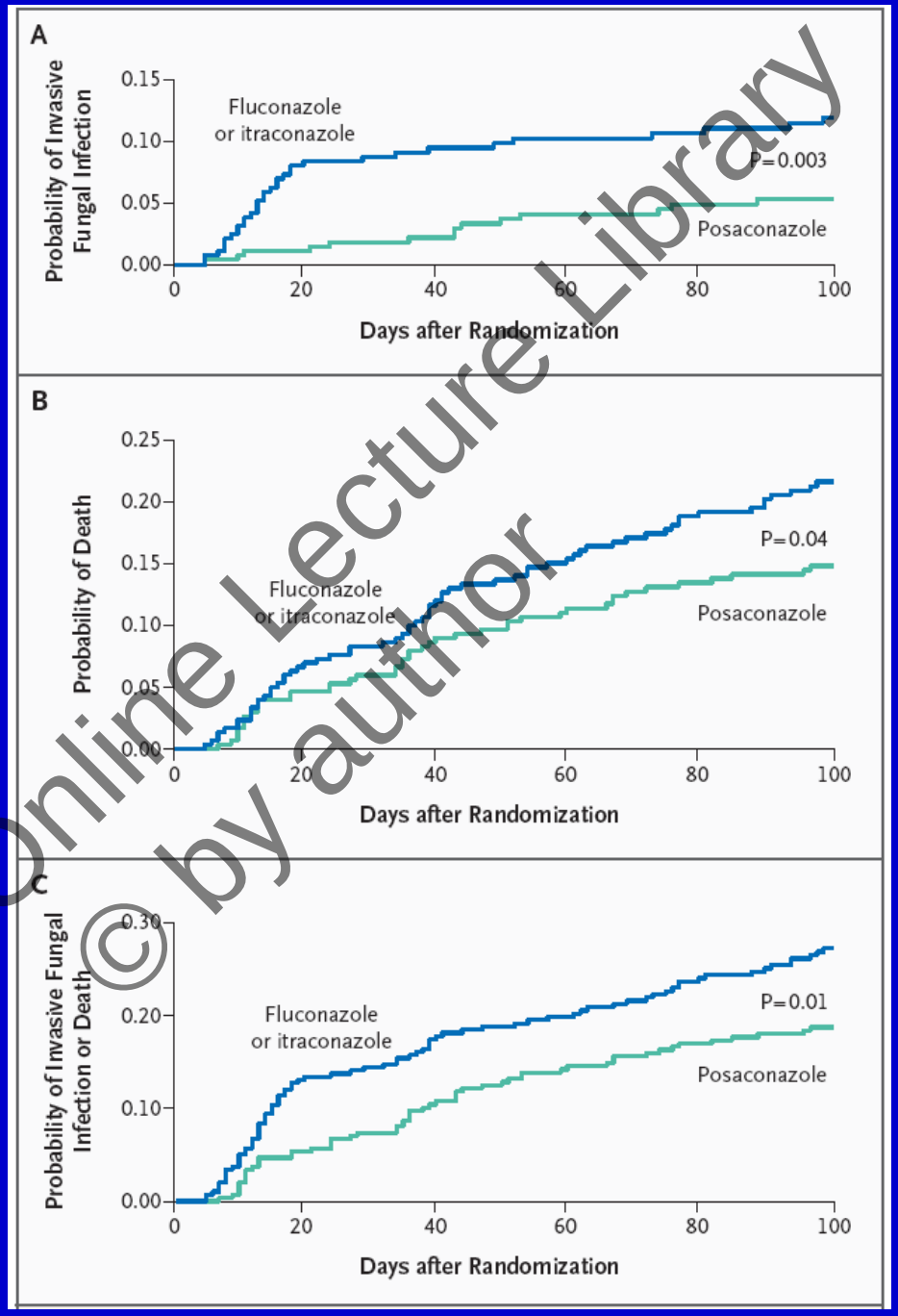
After Casadevall & Pirofski, Infect Immun 1999;67:3703

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

Can we protect patients with immunisation?

Posaconazole prophylaxis in AML



Cornely OA, *NEJM* 2007; 356: 348.

Aspergillus vaccine approaches in the literature

- Conidia, inactivated and live attenuated
  - partially protective, if not killed
- Heat-killed *Saccharomyces cerevisiae,* parenteral and oral
  - partially protective, and broad spectrum
- Asp f3
  - protective, if administered with adjuvant
- Recombinant Asp f2 and derived peptides
  - Immunodominant T cell epitopes were partially protective
- Beta-glucan-CRM197 conjugates
  - protective in mice challenged with *Candida albicans*
- Dendritic cell vaccines, pulsed with Asp f9, IL12 .
  - partially protective, requiring live cell infusion

An Aspergillus vaccine for what?

1. Prevent invasive disease? ✓
2. Improve outcomes of invasive disease (immune augmentation)?
3. Abolish allergic aspergillosis? [Immunotherapy]

Possible endpoints for a phase 3 Aspergillus vaccine study

- All cause mortality (likely to be insensitive)
- Aspergillosis-free survival (useful, if IA can be excluded)
- Cases of IA (optimal if IA can be reliably diagnosed)
- Time to development of IA (unlikely to be a regulatory endpoint, and implies loss of protection over time)
- Surrogate marker of IA as key endpoint (blood GM or PCR) (applies only to haematology patients; perhaps not specific enough; GM not species specific)

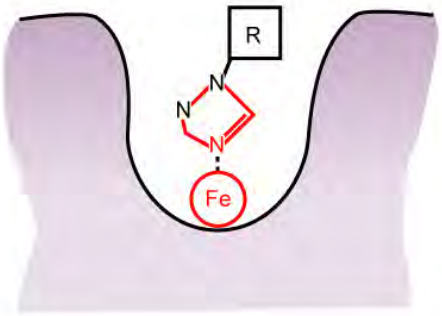
Confounders of endpoints for a phase 3 *Aspergillus* vaccine study

- Antifungal prophylaxis
- Empiric antifungal therapy
- Mixed fungal or bacterial infection
- non-*fumigatus Aspergillus* infection (if *fumigatus* only)
- Ethnic/genetic response characteristics to the vaccine
- Atopic status, including asthma
- Severity and persistence of immunosuppression versus resolution of immunosuppression
- Exaggerated immune response to IA with IRIS-like syndrome, in some vaccinees
- Others

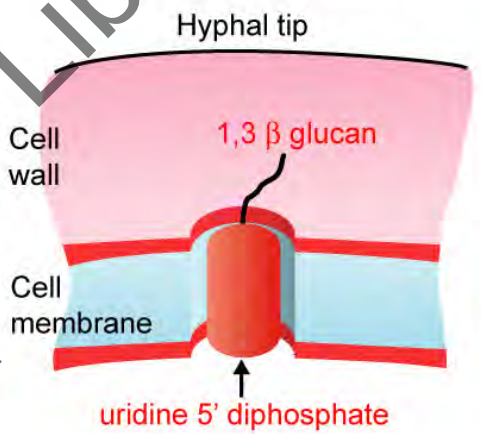
New antifungal agents and resistance

Current antifungal classes

Triazoles

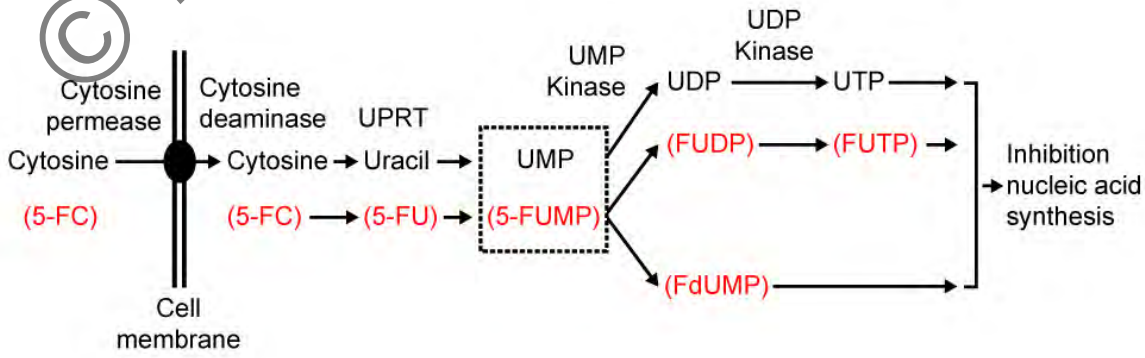


Echinocandins

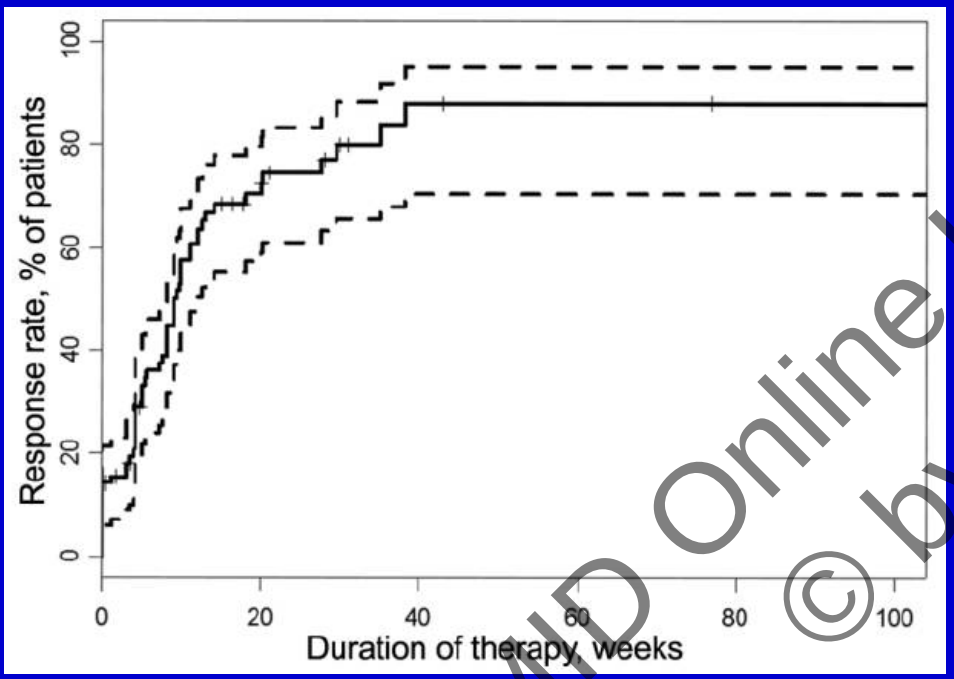


Polyenes

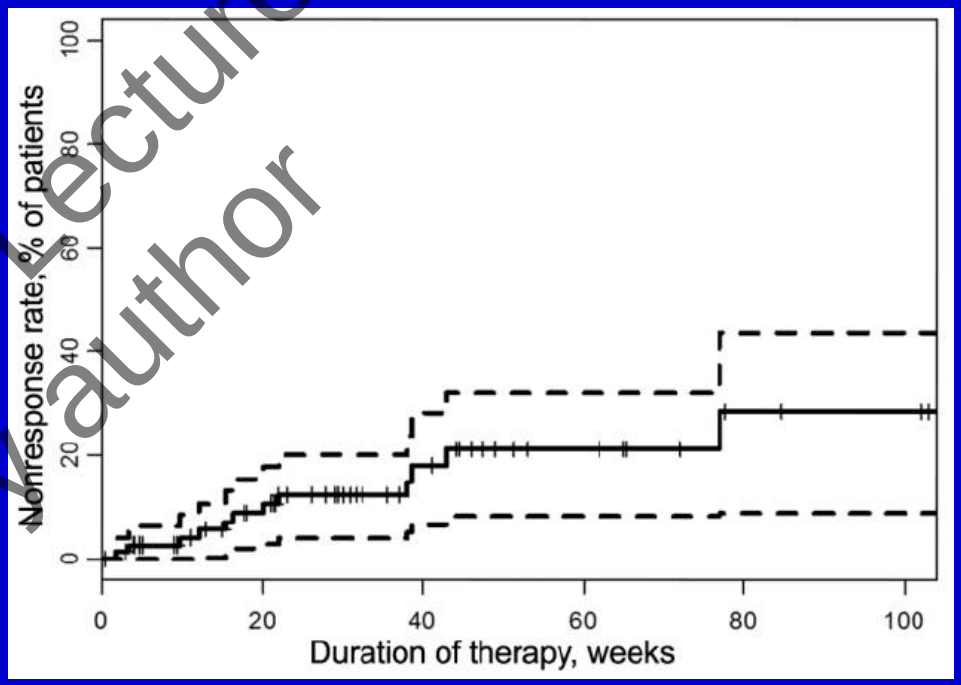
Flucytosine



Posaconazole for chronic pulmonary aspergillosis



Response



Failure and death

Box 1. Priorities for development of novel antifungal agents for the treatment of invasive fungal infections

- Oral compound with activity against all common *Candida* species (including triazole-resistant strains).
- Parenteral or oral compounds with activity against *Cryptococcus neoformans* and penetration into the central nervous system.
- Parenteral and oral compounds with activity against *Aspergillus* spp., including triazole-resistant species. Ideally, compounds should have few drug interactions, and should be safe in patients with renal or hepatic impairment.
- Parenteral and oral compounds with activity against rare, but medically important moulds (e.g. Mucorales, *Scedosporium* spp.).
- Oral agent(s) for the treatment of chronic pulmonary and allergic aspergillosis, with few drug interactions (especially with corticosteroids) and favourable intrapulmonary pharmacokinetics.
- Development of novel formulations of existing compounds that have a more favourable pharmacokinetic properties (e.g. enhanced oral bioavailability)
- Formulations that enable novel uses of existing compounds (e.g. aerosolisation)

Research funding for fungal diseases in the UK

Wellcome	MRC
1.4%	2.5%

from the total spent over the last five years on immunology and infectious disease research

Conclusions

- Clear cut progress in many aspects of medical mycology in last 25 years, especially new drugs, some diagnostics and resistance
- Impact of fungal infection on patients, other than survival, not assessed with standard tools
- Better risk assessment tools, including genetic markers, will allow better protection strategies
- More work required on vaccines
- New antifungals required because of azole resistance, with prospect of routine combination therapies, especially for longterm therapies
- Chronic, relapsing and allergic fungal disease are BIG problems that need more attention