MODERN DIAGNOSIS OF FUNGAL INFECTIONS

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MODERN DIAGNOSIS OF FUNGAL INFECTIONS

Scope of the problem

Conventional diagnosis

Modern diagnosis

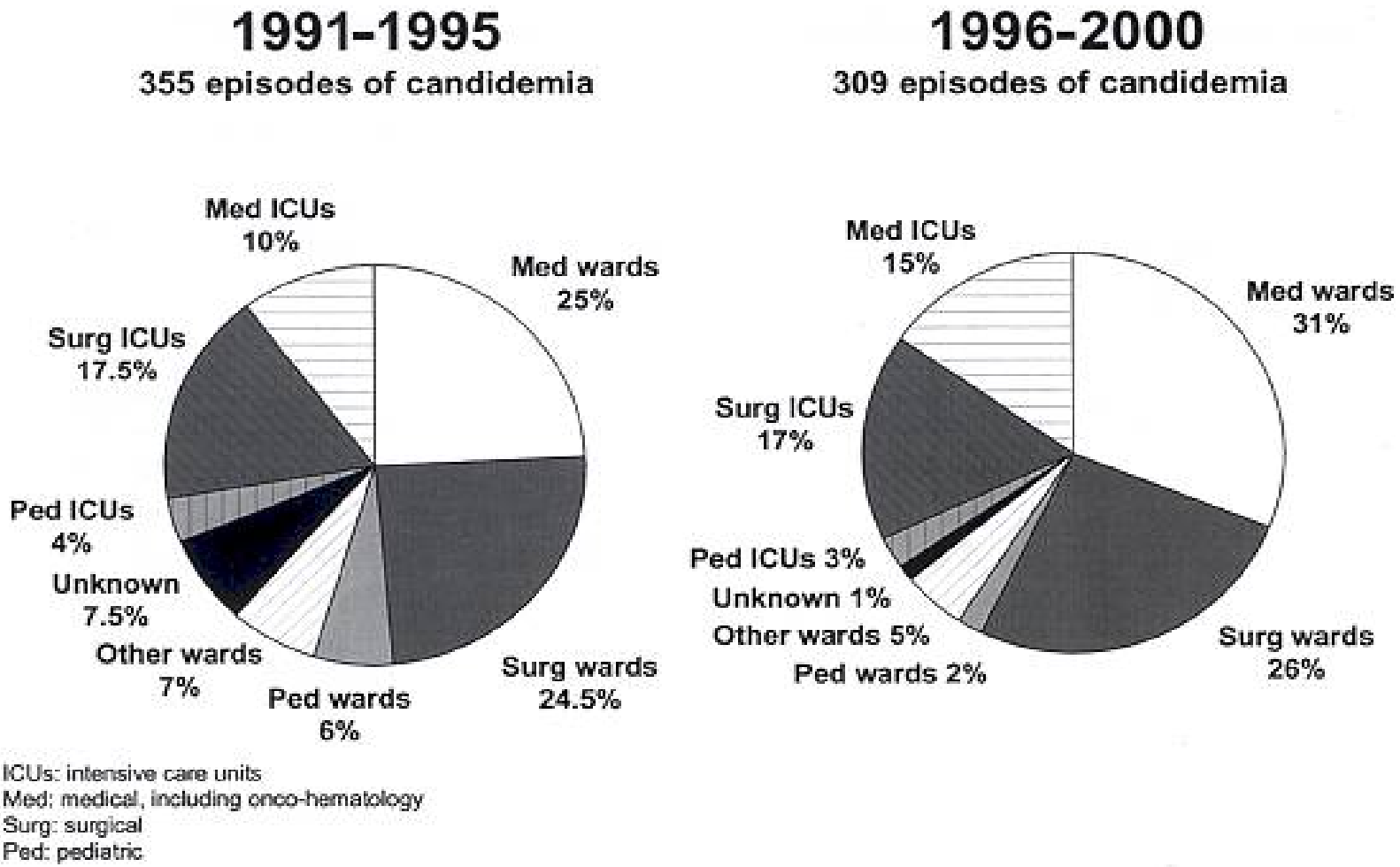
Perspectives

MODERN DIAGNOSIS OF FUNGAL INFECTIONS

Invasive mycoses

Candida		70–90%
Aspergillus		10–20%
All others	*Cryptococcus*	~5%
	Mucorales	
	Fusarium	
	Scedosporium	
	> 100 other	

Epidemiology – Contribution of ICUs



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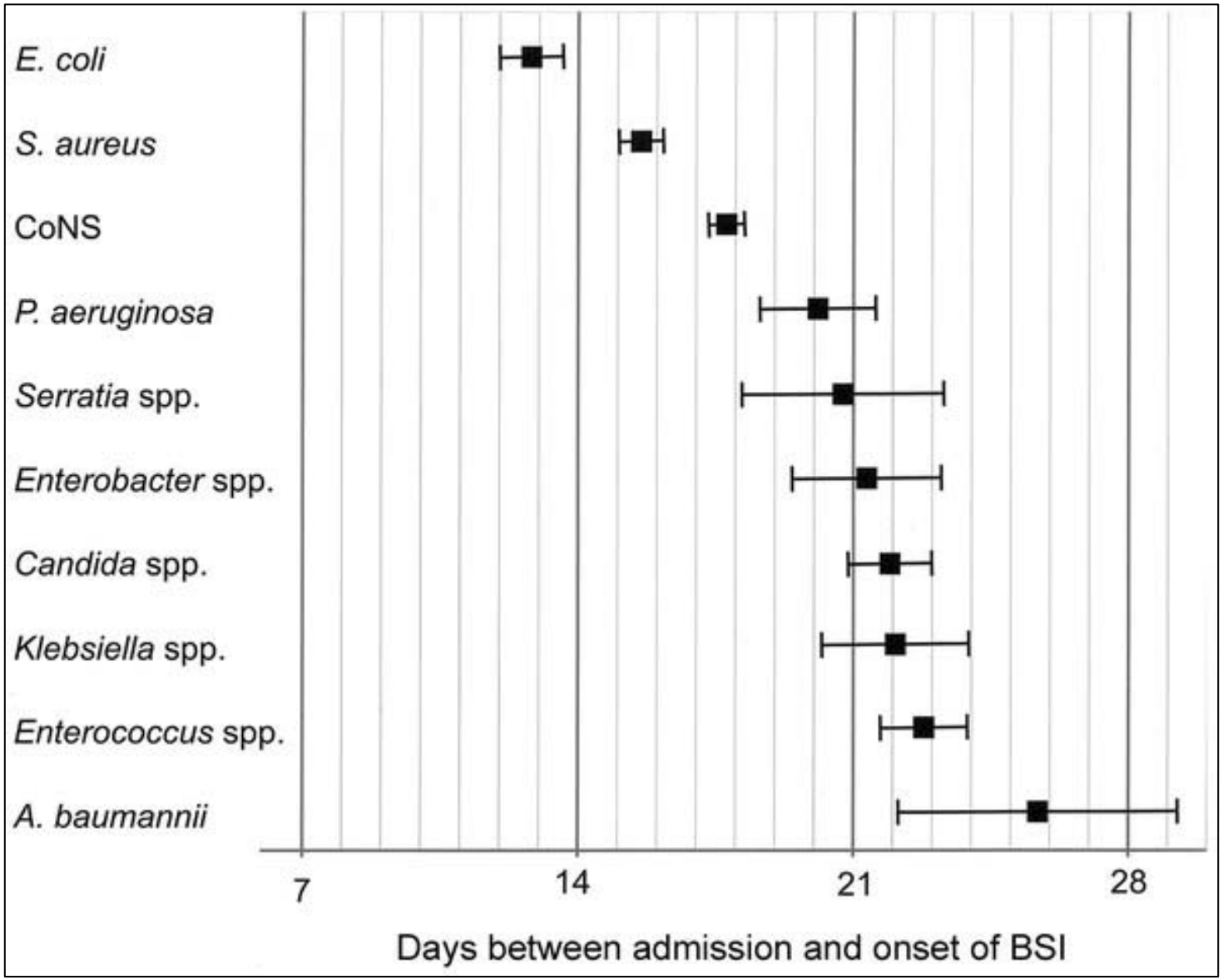

Wisplinghoff H, et al. *Clinical Infectious Diseases*. 2004; 39: 309

Clinical Diagnosis

Problematic

Clinical presentation variable and non specific

fever	80%
leukocytosis	50%
chorioretinitis	25%
endophtalmitis	10-20%

skin lesions

muscle abscesses

septic arthritis

high grade candiduria in non catheterized patients

signs of multi-organ failure

MODERN DIAGNOSIS OF FUNGAL INFECTIONS

Laboratory diagnosis

Candida – Conventional methods

- Direct examination of clinical samples
- Culture
  - blood
  - other sterile body sites
  - urines
  - other sites (dd colonisation-infection)

⊖ insensitive
slow

⊕ identification to species level
antifungal susceptibility testing

- Histology — biopsies (liver, skin)

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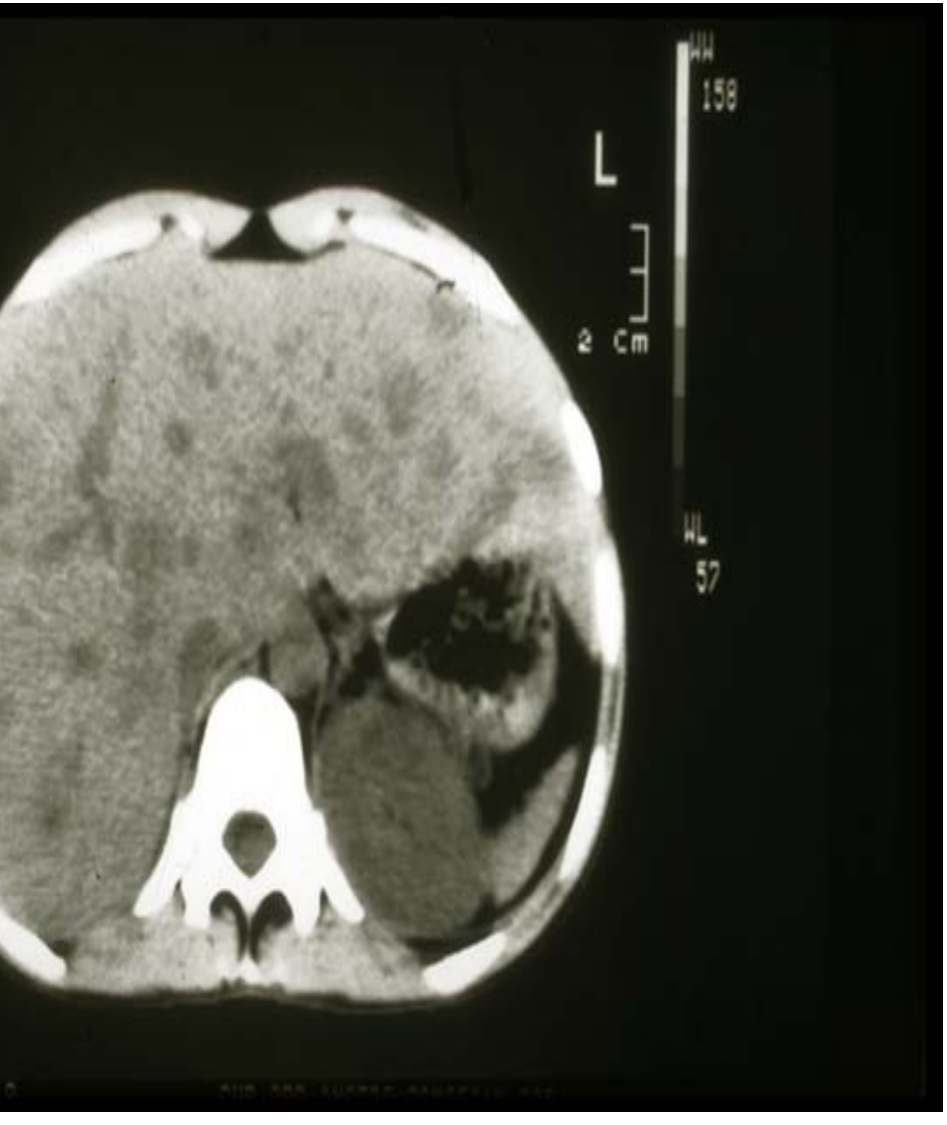
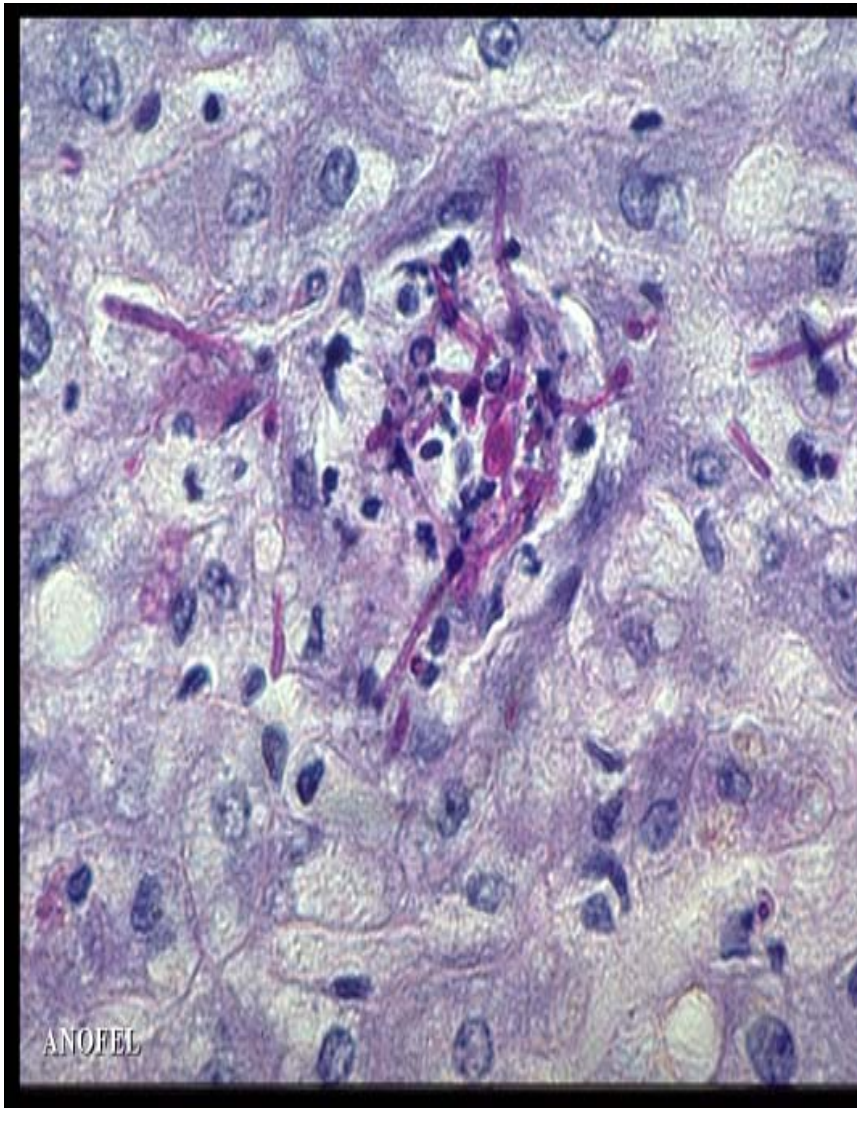
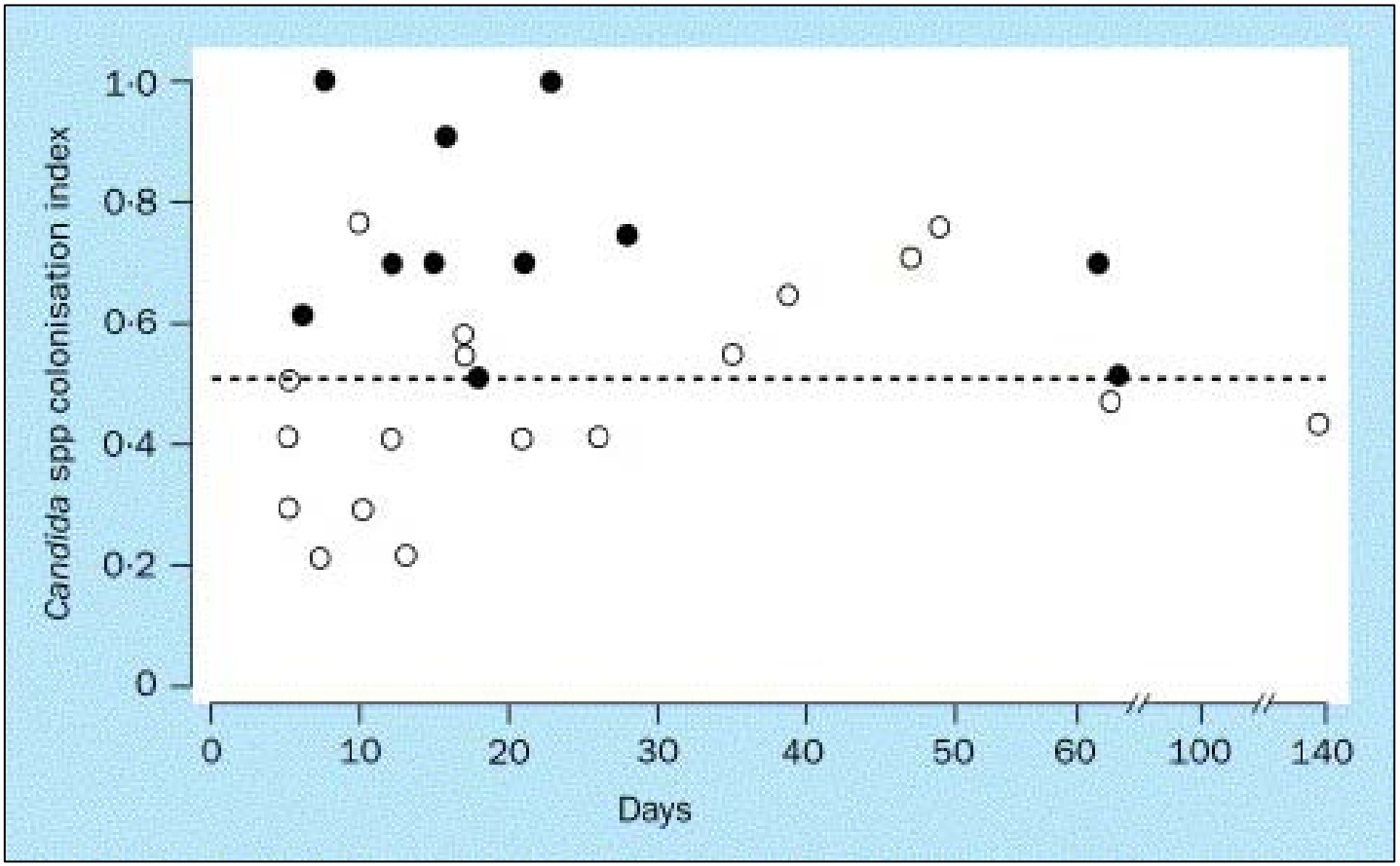




Risk factors Colonisation

Spread from abdominal cavity to other body site
Heavy or increased growth from peritoneal cavit
High amounts in stool
Multiple site colonization
Patient specific strain carriage
Colonisation rate at entry 5-15%
during prolonged ICU stay 50-85%

Risk factors *Candida* colonisation



Colonisation index: ratio of the nb of body sites colonis
to the total nb of body sites culture

Eggimann P. et al., *Lancet Infectious Diseases* 2003; 3: 685.

nvasive Candidiasis (IC) in surgical ICU patients
Association with selected anatomic sites colonizatio

182 SICU pts.

2851 surveillance fungal cultures from 5 sites

Site(s) of colonization	% IC if positive	colonization negative
Urine	13.2%	2.8%
respiratory tract	8.0%	1.2%
rectum/ostomy	8.4%	0%
urine and respiratory		0%

Magill SS et al., *Diagnostic Microbiology and Infectious Disease* 2006; 55: 293.

Laboratory diagnosis

Candida – indirect tests

- antigen-antibodies
- fungal DNA detection

Mostly evaluated in onco-hematology patients

Very few studies in ICU patients

Very few commercially available tests

Diagnosis of invasive *Candida* infections

Antigen based tests

	detection limit	
Mannan	0.1 μg/mL	specific to *Candida* spp
β-1-3 D glucan	0.1 pg/mL	non specific to *Candida* sp

Diagnosis of invasive *Candida* infections
Mannan (+ antimannan)

quency of testing
-3 times/week
g peak very short

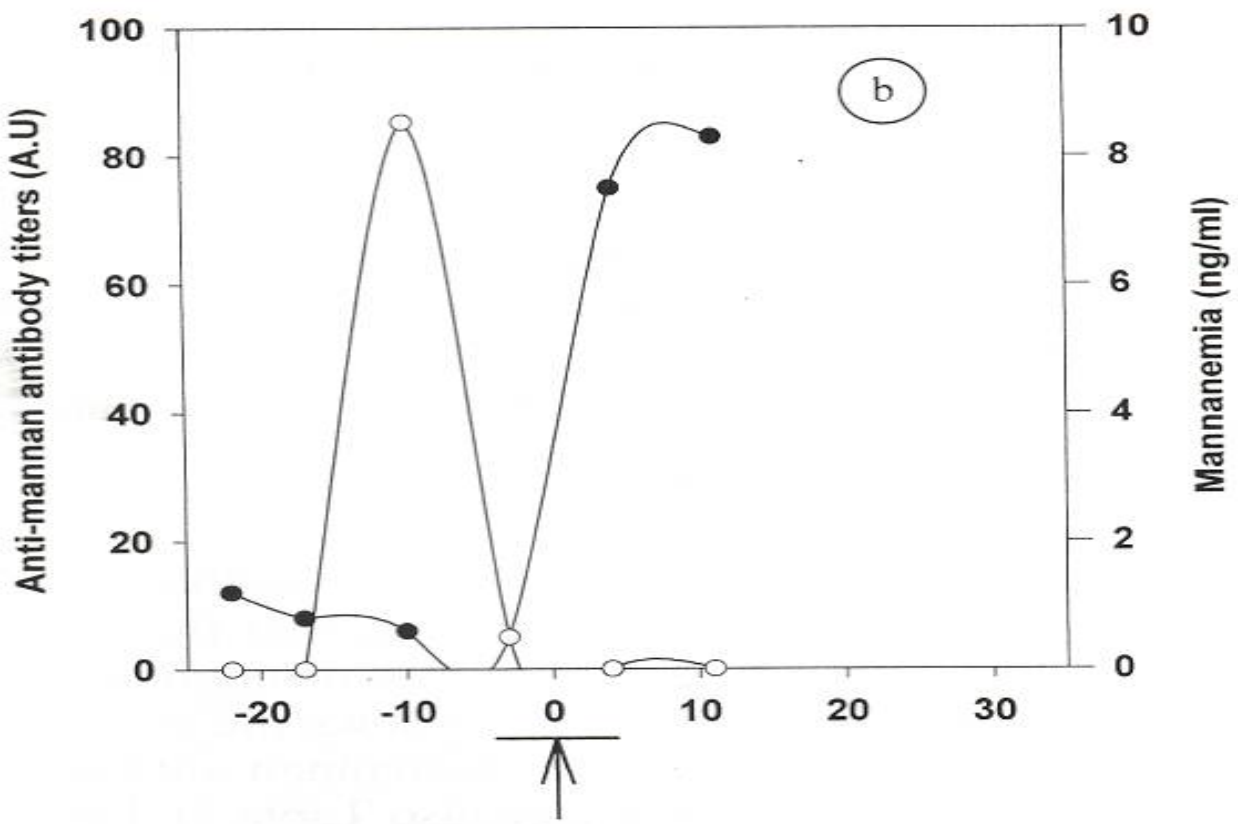


FIG. 4. Examples of kinetic evolution of antigenemia (○) and antima
antibody response (●) detected by EIA. Patients 39 (a) and 43 (b) had syst
candidiasis. The arrow marks the date of mycological isolation of *C. alb*
from blood and a drain. The curves are drawn by using the interpolate regres

ndid, *JCM*
99; 37: 1514.

Diagnosis of invasive *Candida* infections

Mannan (+ antimannan)

<u>Sensitivity factors</u>

main variables:

- type of disease candidemia in:
  - oncohematology
  - ICU patients

  hepatosplenic candidiasis
- species of *Candida*
- stage of the disease (antibodies)
- frequency of testing (per test or per episode evaluation)

Mannan screening in ICU patients

Prospective study of 105 ICU patients (>7 days in ICU
Screening 1x/week

IC n=10 (2 proven, 3 probable)

Colonization rate : 70%

Mannan positive in 60% pt with IC
43% pt colonized
25% pt without colonizatior

20% of false positive results !

lbara A.S. et al., *J Mycol Med* 2004; 14: 34-42.

Multicenter Clinical Evaluation of β-D-glucan Assa

163 patients with IFI, 170 without - 1 sample taken within
72 hours after diagno

107 patients with proven candidiasis

sensitivity 81.3%

All patients	sensitivity	70%
	specificity	87%
	PPV	83.8%
	NPV	75.1%

β-D-glucan for diagnosis of IFI in patients with acute infections

	BG assay positive	
	(Digby 2003)	*(Pickering 2005)*
IFI confirmed	96% (25/26)	87% (13/15)
Bacteremia	73% (8/11)	56% (14/25)
No infection	22% (2/9)	

Low positive predictive value

High negative predictive value (≥ 95%)

Pickering JW et al., *J. Clin Microbiol* 2005; 43: 5957.
Digby J et al., *Clin Diag Lab Immunol* 2003; 10: 882.

Molecular based tests

DNA detection (PCR)

Single species/genus versus panfungal

High sensitivity (1-10 fg of DNA)

Applied to various body fluids : blood, serum, CSF, BAL

Quantification possible (response to antifungal therapy, differentiation colonization-infection)

No standardized (commercial) assay

→ no prospective multicenter large studies

Aspergillosis

Clinical signs/symptoms are non specific

- fever
- dyspnea
- non productive cough
- hemoptysis
- chest pain
- pleural rib

In a proper host
rapidly progressive

Aspergillus – Conventional diagnosis

- direct examination
  of tissue
  of indirect clinical specimens (sputum, BAL)

	sputum/BAL	tissue
unstained	wet prep ± KOH	
routine stains	Gram	HE
fungal stains	GMS, PAS	GMS, PAS
fluorescent dyes	Calcofluor white Uvitex 2B Blankophor	

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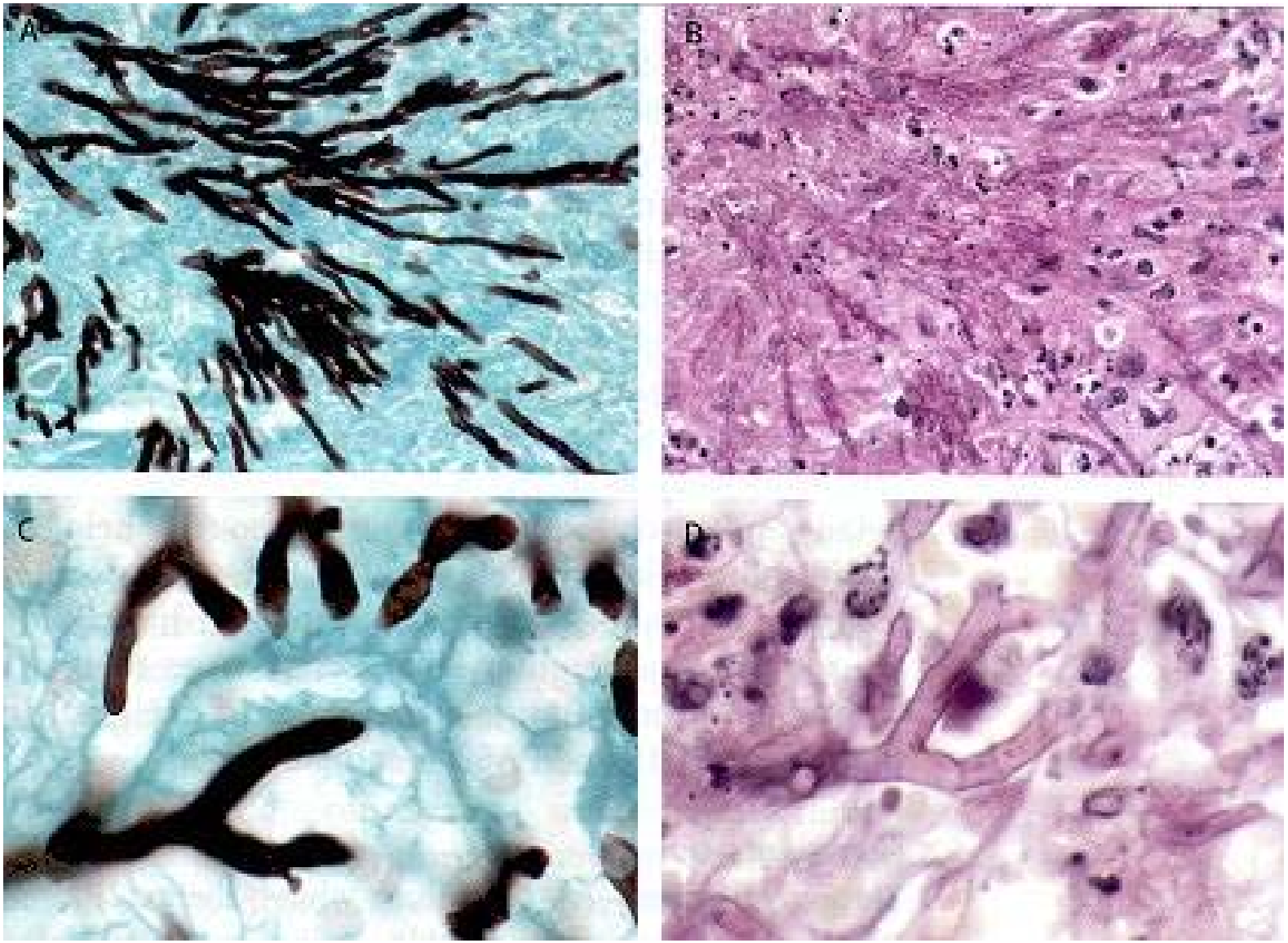

In: Hope et al., *Lancet Infectious Diseases* 5: 609, 2005

Aspergillus – Culture

Media

- **Isolation :** Sabouraud (+ antibiotics)
  blood agar, chocolate agar
- **Identification :** malt-extract, corn-meal aga
  Czapek agar

Incubation

temperature	25-30°C	→ improvement ?
athmosphere	aerobic	
duration	2-6 weeks	

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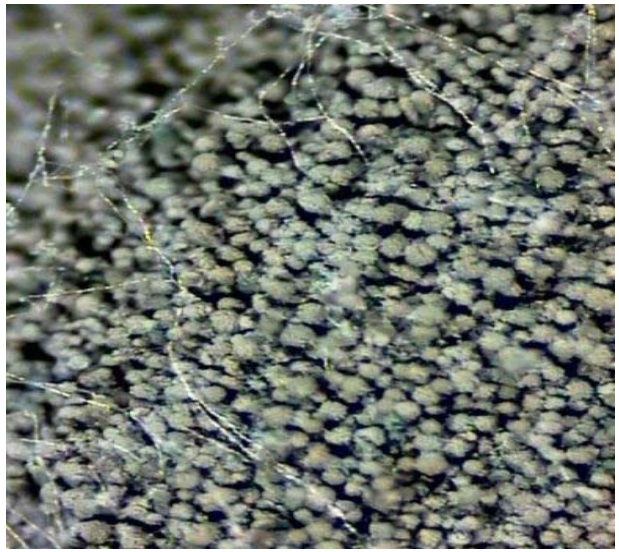

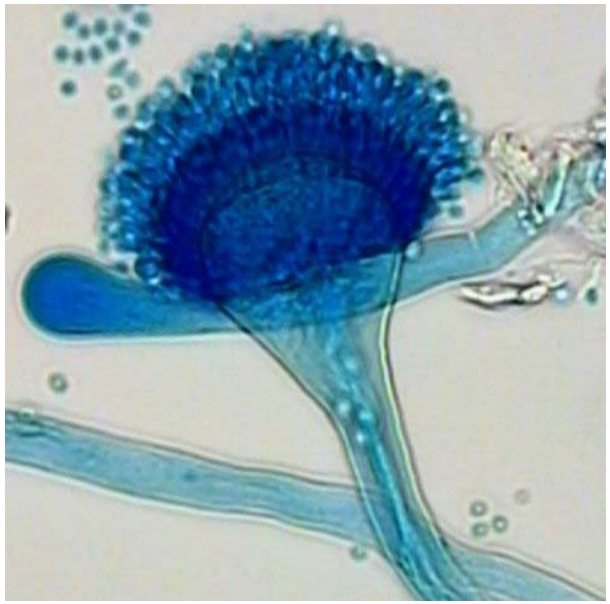

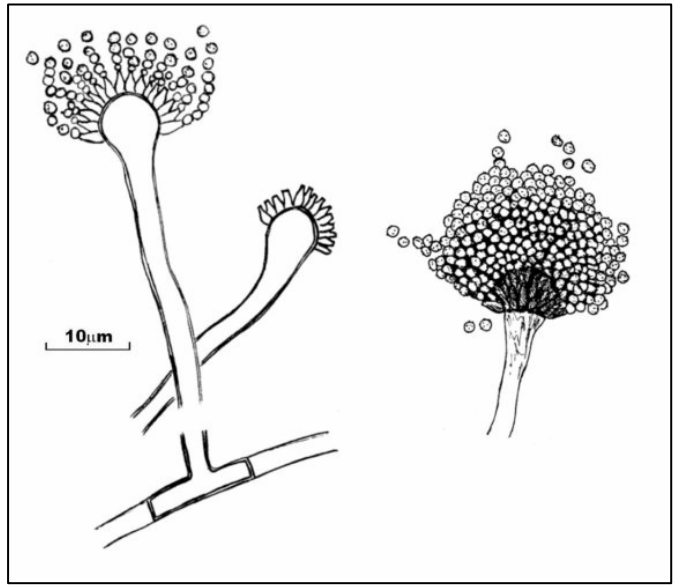

In: Andreoni et al., *Medical Mycology Atlas*.

Yield of culture for molds in histopathologic positive samples

	n=	culture	positive
Autopsy samples	23	12	52%
Surgical or biopsy tissue	30	9	30%

Tarrand J.J. et al., *Am J Clin Pathol* 2003; 119: 854

Positive predictive value of a positive sputum culture for IA

Highly variable (15-77%)

Depends on host factors

allo BMT	60%
leukemia + neutropenia	70-80%
SOT	20-60%
HIV/AIDS	14-20%
Corticosteroids	20%

Perfect et al. (MSG), *Clin Infect Dis* 2001; 33: 1824
Yu et al., *Am J Med* 1986; 81: 249
Horvath and Dummer, *Am J Med* 1996; 100: 171

Aspergillus
Antigen based-test – Galactomannan

t.	Gm positive in pt. with IA*	Sens.	GM negative in pt. without IA	Spec.	Cut-off	Ref.
Allo-HSCT	17/18	94%	72/73	99%	1.5 (2x)	Maertens *JID* 2002
FN	31/98	32%	607/640	95%	1.5 (1x)	Herbrecht *JCO* 200
FN	8/17	47%	134/143	94%	1.0	Becker *BJH* 2003
FN	48/53	91%	700/744	94%	1.5 (>1x)	Sulahian *Cancer* 2
BMT	13/24	54%	32/43	74%	1.0	Marr *JID* 2004

proven or probable cases of aspergillosis only

Comparison of invasive aspergillosis in neutropenic and non neutropenic patients

Single institution during 6 y
88 cases (12 proven, 52 probable, 24 possible)

	neutropenia +		neutropenia -
n=	59%		41%
mortality	60%	<	89%
BAL positive	58%	<	85%
Galactomannan positive	64%		65%
anti-aspergillus antibodies	6%	<	48%

Cornillet et al., *Clinical Infectious Diseases* 2006; 43: 577.

urrent recommendations

ew tests

- Complementary to conventional methods
- Used as screening methods in targeted patien
- High NPV → reduction of empirical therapy
- High PPV → early targeted preemptive therap
- Cost-efficiency, impact on outcome :
  data still pending