What have we learnt from clinical trials in invasive candidiasis?

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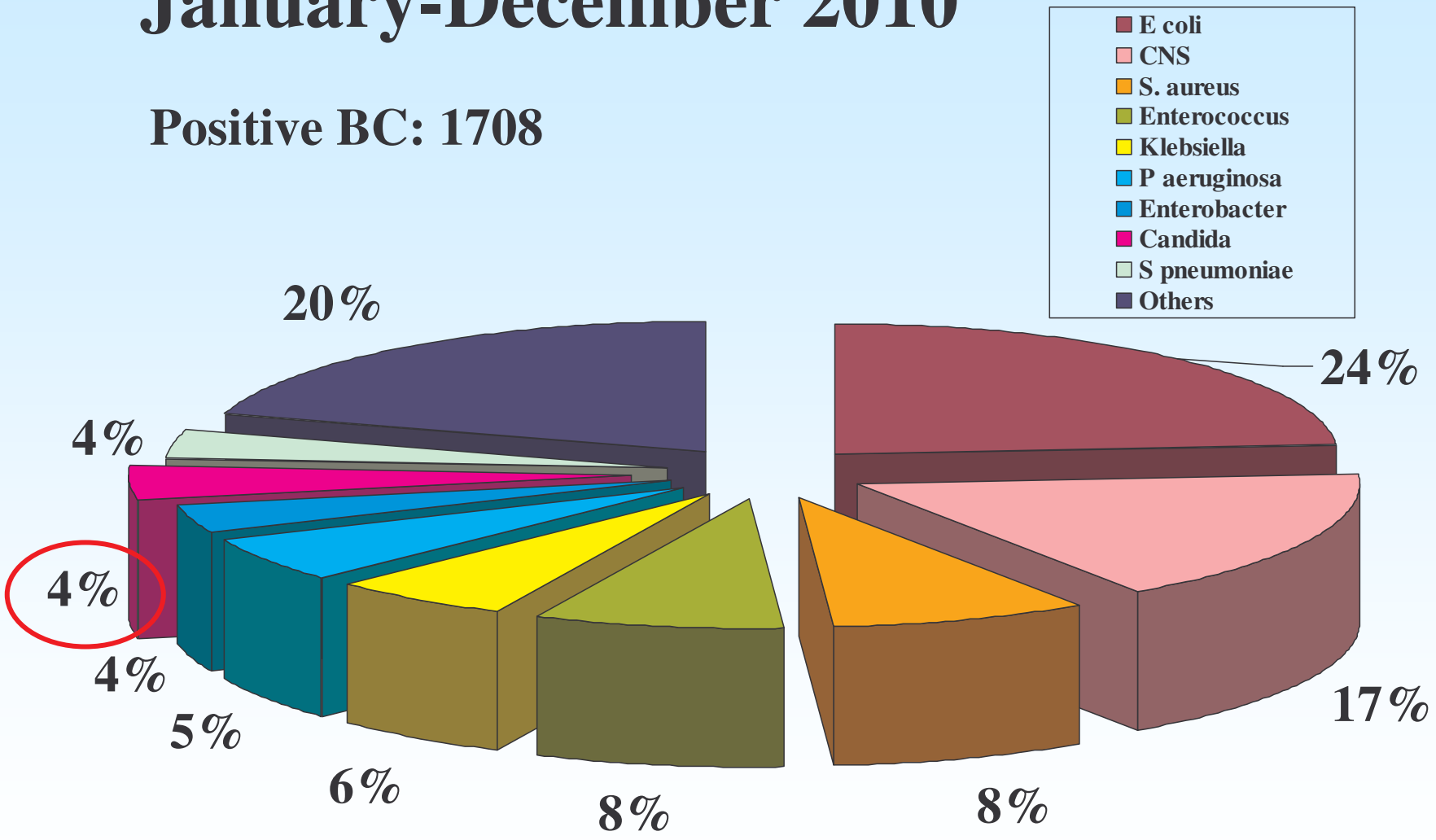
Epidemiology

- *Candida* spp: most common cause of invasive fungal infections (70-90 % mycoses)
- Causes of bloodstream infection: Candida is 4th in frequence (USA) and 7th Europe
- Candidemia incidence: higher in ICUs and patients suffering cancer
- Mortality ~ 40 % (mainly immunocompromised patients)

January-December 2010

Positive BC: 1708



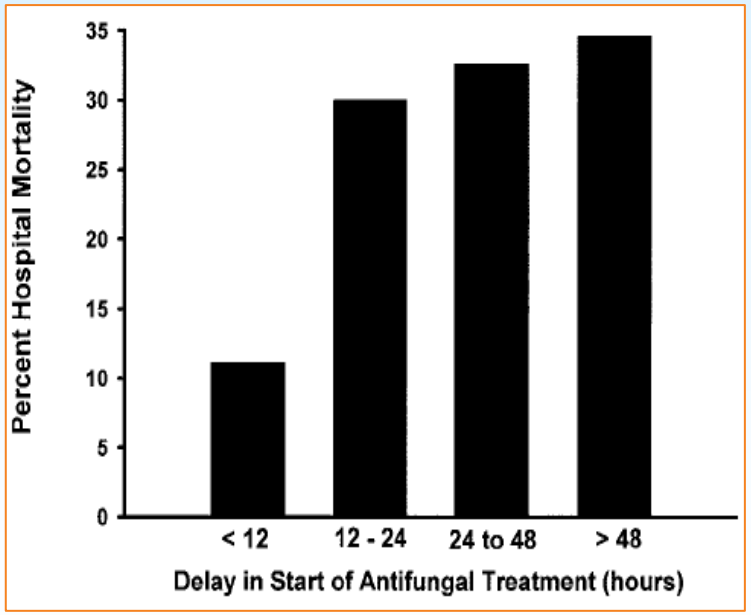
Delaying the Empiric Treatment of *Candida* Bloodstream Infection until Positive Blood Culture Results Are Obtained: a Potential Risk Factor for Hospital Mortality

Matthew Morrell,[1] Victoria J. Fraser,[2] and Marin H. Kollef[1]*

Pulmonary and Critical Care Division[1] and Division of Infectious Diseases,[2] Washington University School of Medicine, St. Louis, Missouri 63110

→ Administration of empiric antifungal treatment 12 h after a positive blood sample for culture is drawn: greater mortality

→ More rapid diagnostic techniques are required



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2005, p. 3640–3645

Why is it so difficult to diagnose a systemic candidiasis?

1.- Low sensitivity of the *gold standard* diagnostic technique

20/94 cases of IC demonstrated at autopsy had + blood cultures

(Kami M et al, Br J Haematol 2002 Apr;117(1):40-6)

2.- When positive BC take at least 48 h to detect Candida growth

3.- Clinical suspicion: lack of suggestive specific signs and symptoms

4.- Endogenous origin → continuum from colonisation to infection: when does the former finish and the latter begin?

Micology laboratory aid to diagnosis of fungal infections

- Conventional methodologies
  - Direct simple examination (Ex: *Calcofluor* staining)
  - Culture (lack sensitivity and slowness of turnaround times...)

- Non-conventional methodologies
  - Antigen detection
  - Antibody detection
  - Genetic techniques

Common advantatge: No viability of the microorganism is required

Non-conventional methodologies

- **Antigen detection:**

  1.- Mannan

  2.- β-(1-3) Glucan

  3.- Galactomannan

  4.- *C neoformans* capsular antigen

- **Antibodies detection:**

  1.- anti-mannan

  2.- anti-*C albicans* germ tube

- **Genetic techniques:**

  1.- Candida PCR

  2.- *Aspergillus* PCR

Non-conventional methodologies

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  1.- Candida PCR

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Combined detection of mannanaemia and anti-mannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic *Candida* species

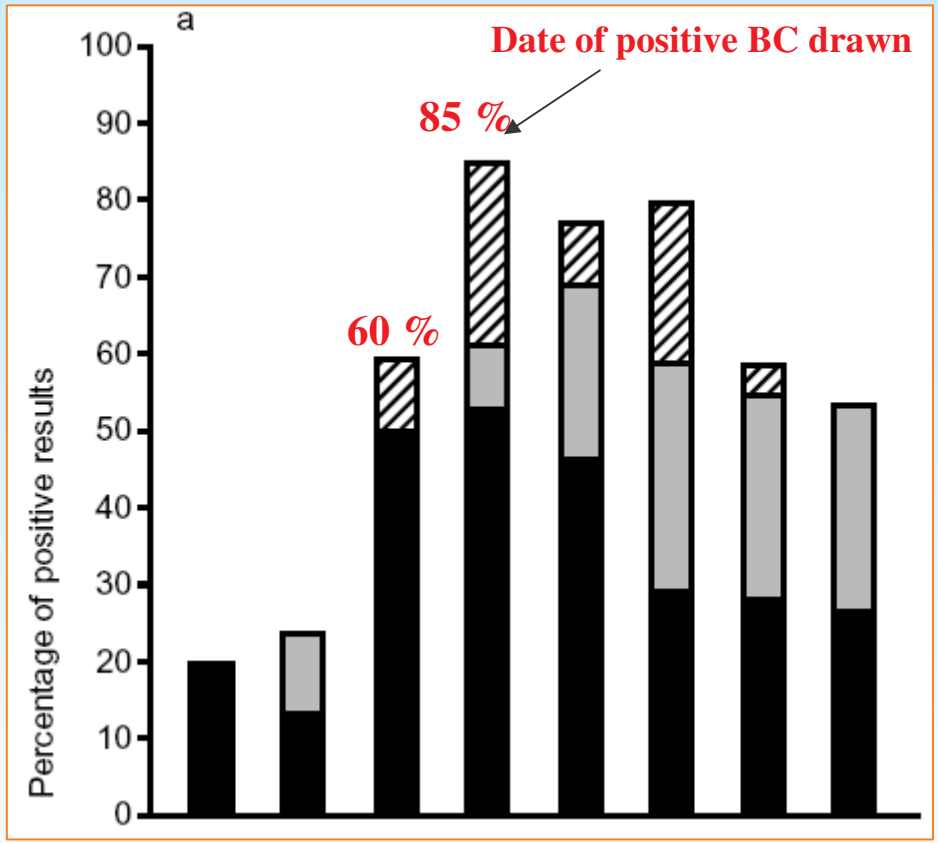
BOUALEM SENDID*†, JEAN LOUIS POIROT§, MARC TABOURET‡, ALAIN BONNIN‖, DENIS CAILLOT**, DANIEL CAMUS† and DANIEL POULAIN*†

→ 204 serum samples analysed, belonging to 63 patients with proven candidiasis

→ All *C albicans* infected patients had at least 1 positive serum for one or both tests
(S: Mn+Anti-Mn: 100 %)

→ At least 1 serological test positive before yeast growth in 60 % of patients

J. Med. Microbiol. — Vol. 51 (2002), 433–442

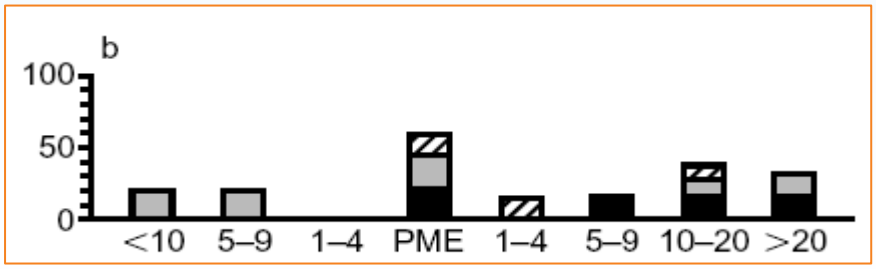


Positive A-Mn + or Mn serums

Positive A-Mn serums

Positive Mn serums

C albicans, C glabrata, C tropicalis



C parapsilosis, C krusei, C kefyr

Prospective evaluation of mannan and anti-mannan antibodies for diagnosis of invasive *Candida* infections in patients with neutropenic fever

Michael Ellis,[1] Basel Al-Ramadi,[2] Roos Bernsen,[3] Jorgen Kristensen,[4] Hussain Alizadeh[4] and Ulla Hedstrom[5]

Journal of Medical Microbiology (2009), **58**, 606–615

→ 12 proven/probable invasive candidiasis, 50 febrile neutropenia

→ optimal overall performance achieved: two consecutive samples for both Mn and Anti-Mn:
S: 73 %, E: 80 %, PPV: 36 % and NPV: 95 %

→ High NPV, particularly useful in excluding IC

The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia

Małgorzata Mikulska[1*], Thierry Calandra[2], Maurizio Sanguinetti[3], Daniel Poulain[4], Claudio Viscoli[5], the Third European Conference on Infections in Leukemia Group

In summary:

→ Mannan + anti-mannan detection tests improve the chances of early detection of candidiasis due to ***C albicans C glabrata, C tropicalis*** (S to predict ~ 60 %)

Inconveniences:

→ Other species (*C parapsilosis, C krusei)*: low sensitivity

→ ELISA tests: time consuming and expensive

Non-conventional methodologies

- **Antigen detection:**

  1.- Mannan

  2.- β-(1-3) Glucan

  3.- Galactomannan

  4.- Capsular antigen of *C neoformans*

- **Antibodies detection:**

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  1.- Candida PCR

  2.- *Aspergillus* PCR

β-(1-3) Glucan

Major cell wall component of medically important fungi (Candida, Aspergillus and Pneumocystis)

Its detection in blood and normally sterile site body fluids may indicate an invasive fungal infection

Panfungal technique included in EORTC/MSG diagnostic criteria for IFI in 2008

Variable	Fungitec G-Test MK	β-glucan Test Wako	B-G Star	Fungitell
Manufacturer	Seikagaku Corporation	Wako Pure Chemical	Maruha Corporation	Associates of Cape Cod
Country	Japan	Japan	Japan	USA
Approval year	1995	1996	2001	2004
Assay method	Kinetic chromogenic	Kinetic turbidimetry	Endopoint chromogenic	Kinetic chromogenic
Sample	Serum or plasma	Serum or plasma	Serum or plasma	Serum
Pretreatment	Alkali	Dilution and heating	Dilution and heating	Alkali
Standard β-glucan	Pachyman	Carboxymethyl-curdlan	Lentinan	Pachyman
Origin of lysate	*Tachypleus tridentatus*	*Limulus polyphemus*	*Tachypleus tridentatus*	*Limulus polyphemus*
Cutoff value, pg/mL	20	11	11	60 or 80
Measurable range, pg/mL	3.9–500	6–600	1.2–120	31.25–500
Turn-around time, min	30	90	30	40

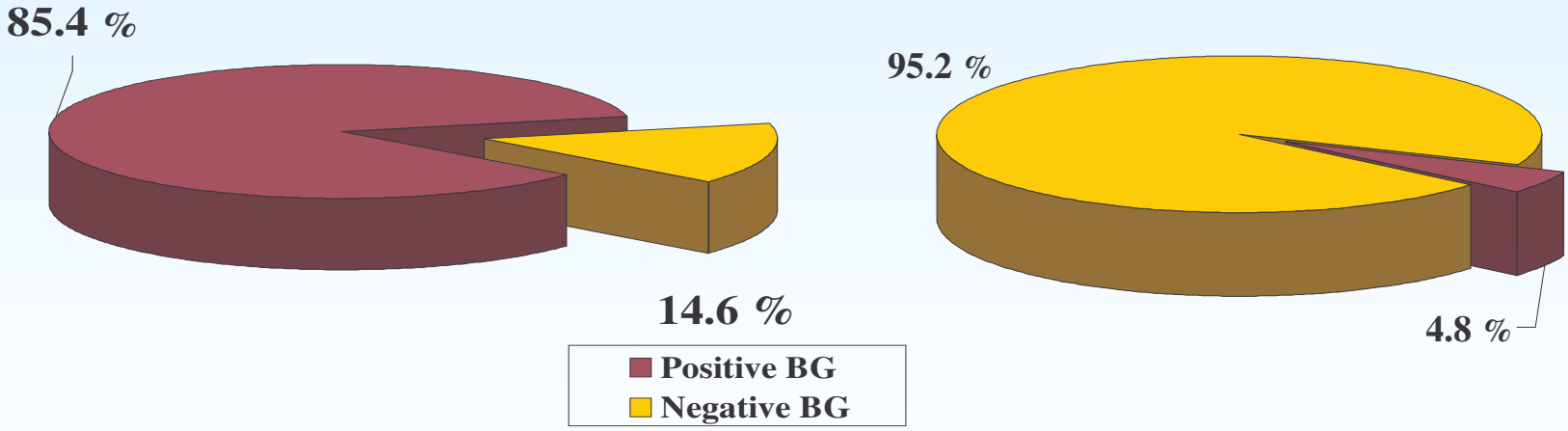
Comparison of 4 commercial kits for the serum (1→3)-β-D-glucan (β-glucan) assay.

Reappraisal of the Serum (1→3)-β-D-Glucan Assay for the Diagnosis of Invasive Fungal Infections— A Study Based on Autopsy Cases from 6 Years

Taminori Obayashi,[1] Kumiko Negishi,[1] Tomokazu Suzuki,[1] and Nobuaki Funata[2]

Efficacy of BG to detect cases of mycoses:

486 autopsies: 41 cases of IFI and 63 patients with no evidence had been analysed for BG



41 cases of IFI

63 without IFI

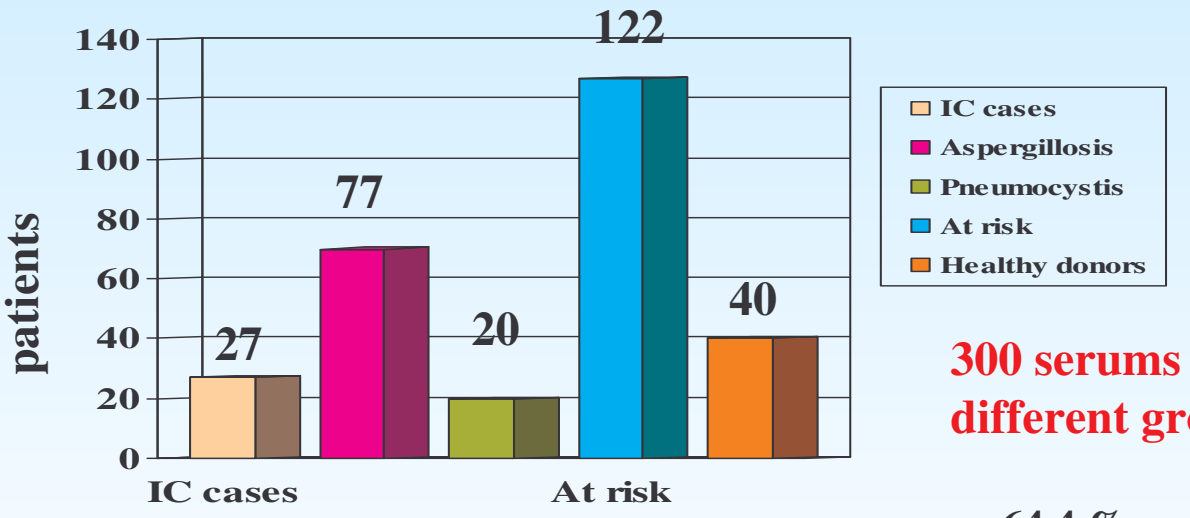
PPV 70.4 % NPV 98 %

CID 2008:46 (15 June) • Obayashi et al.

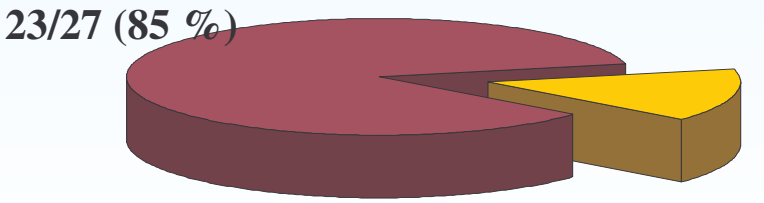
Contribution of the (1→3)-β-D-Glucan Assay for Diagnosis of Invasive Fungal Infections[▽]

Florence Persat,[1]* Stéphane Ranque,[2] Francis Derouin,[3] Annie Michel-Nguyen,[2] Stéphane Picot,[1] and Annie Sulahian[3]

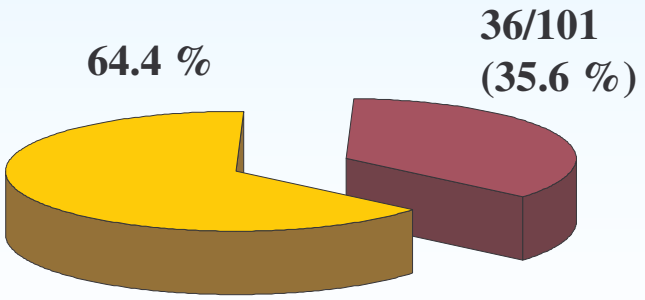
Hospices Civils de Lyon, Hôpital Edouard Herriot, Service de Parasitologie, Mycologie Médicale et Maladies Tropicales, Lyon, France[1]; Laboratoire de Parasitologie-Mycologie, AP-HM Timone, Marseille, France[2]; and Laboratoire de Parasitologie-Mycologie, Hôpital Saint Louis, Paris, France[3]



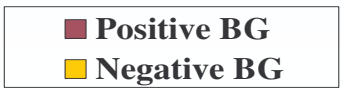
300 serums analysed from different group of patients



27 IC cases



101 at risk patients



JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2008

Diagnostic Performance of the (1→3)-β-D-Glucan Assay for Invasive Fungal Disease

Sophia Koo,[1,2,3] Julie M. Bryar,[1,4] John H. Page,[4] Lindsey R. Baden, [1,2,3] and Francisco M. Marty[1,2,3]

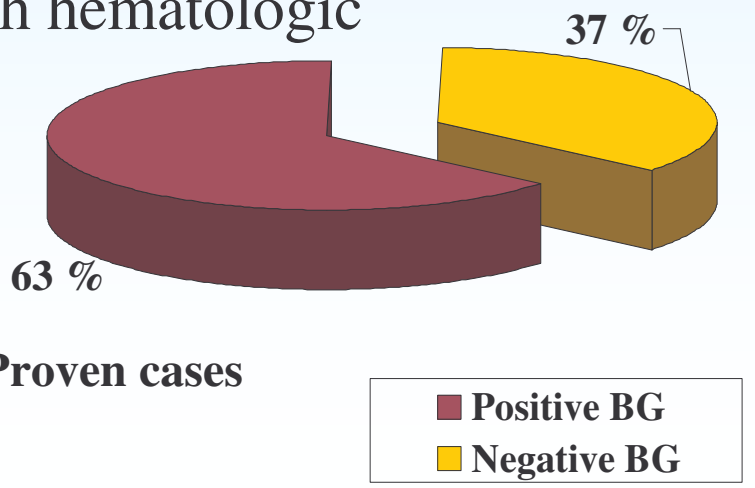
[1]Brigham and Women's Hospital, [2]Dana-Farber Cancer Institute, [3]Harvard Medical School, and [4]Harvard School of Public Health, Boston, Massachusetts

→ Clinical usefulness of BG in a large clinical cohort of patients at risk for IFD

→ 39 proven IC cases: S of the test 1 week before positive BC (to anticipate diagnosis): 63 %

→ less S among patients with hematologic malignancies (51 %)

→ BG level rises more slowly in IC than in IA



CID 2009:49 (1 December) • Koo et al

In summary:

→ Serial serum BG detection appears to be a fair diagnostic adjunct for IFI.

Inconveniences:

→ Panfungal marker: it does not distinguish between other fungal pathogens (Aspergillus, Fusarium...)

→ It is less useful as an early marker of IC than IA

→ Known factors associated with FP results: albumin, intravenous inmunoglobulin, hemodyalisis, antibiotics and concomitant bacteriemia (*P aeruginosa*)

Non-conventional methodologies

- **Antigen detection:**

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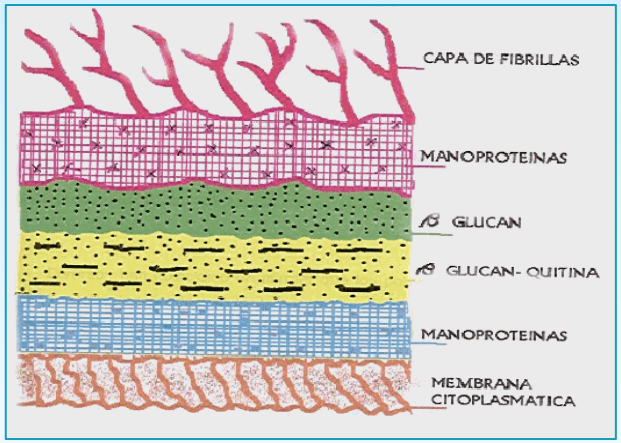
Candida DNA detection by PCR

Delay in the development of fungal PCR techniques…

1.- High complexity of cell wall in fungus → more difficult to disrupt → better DNA extracton systems

2.- Fungus are ubiquitus: risk of contamination while processing clinical samples



Filamentous fungi cell wall

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2005, p. 5122–5128

Comparison of Six DNA Extraction Methods for Recovery of Fungal DNA as Assessed by Quantitative PCR

David N. Fredricks,1,3

*Program in Infectious Diseases*1 *and Pro*

Seattle, Washington, and Department o

University of Wa

Received 12 May 2005/Returned for

Improving molecular detection of *Candida* DNA in whole blood: comparison of seven fungal DNA extraction protocols using real-time PCR

L. Metwally,[1] D. J. Fairley,[1] P. V. Coyle,[1] R. J. Hay,[2] S. Hedderwick,[3] B. McCloskey,[4] H. J. O'Neill,[1] C. H. Webb,[1] W. Elbaz[3] and R. McMullan[1]

Latest technical advances...

1.- Automated DNA extraction systems → higher efficiency with less clinical sample manipulation

2.- Real time PCR that excludes additional detection systems (eletrophoresis, ELISA…) → less clinical sample manipulation (= contamination risk)

3.- Combination of both: better sensitivity, specificity and quicker turnaround times

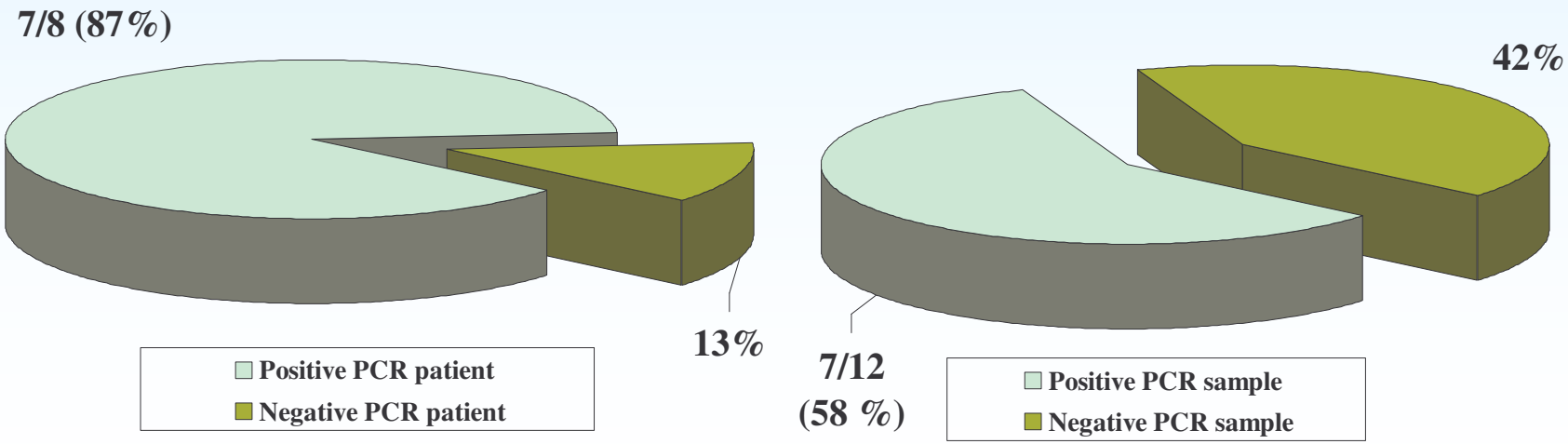
Rapid diagnosis of candidaemia by real-time PCR detection of *Candida* DNA in blood samples

Nele Wellinghausen,[1,2] Dunja Siegel,[1] Juliane Winter[1]† and Susanne Gebert[1]†

→ 384 at risk patients for IC (hematology and ICUs)

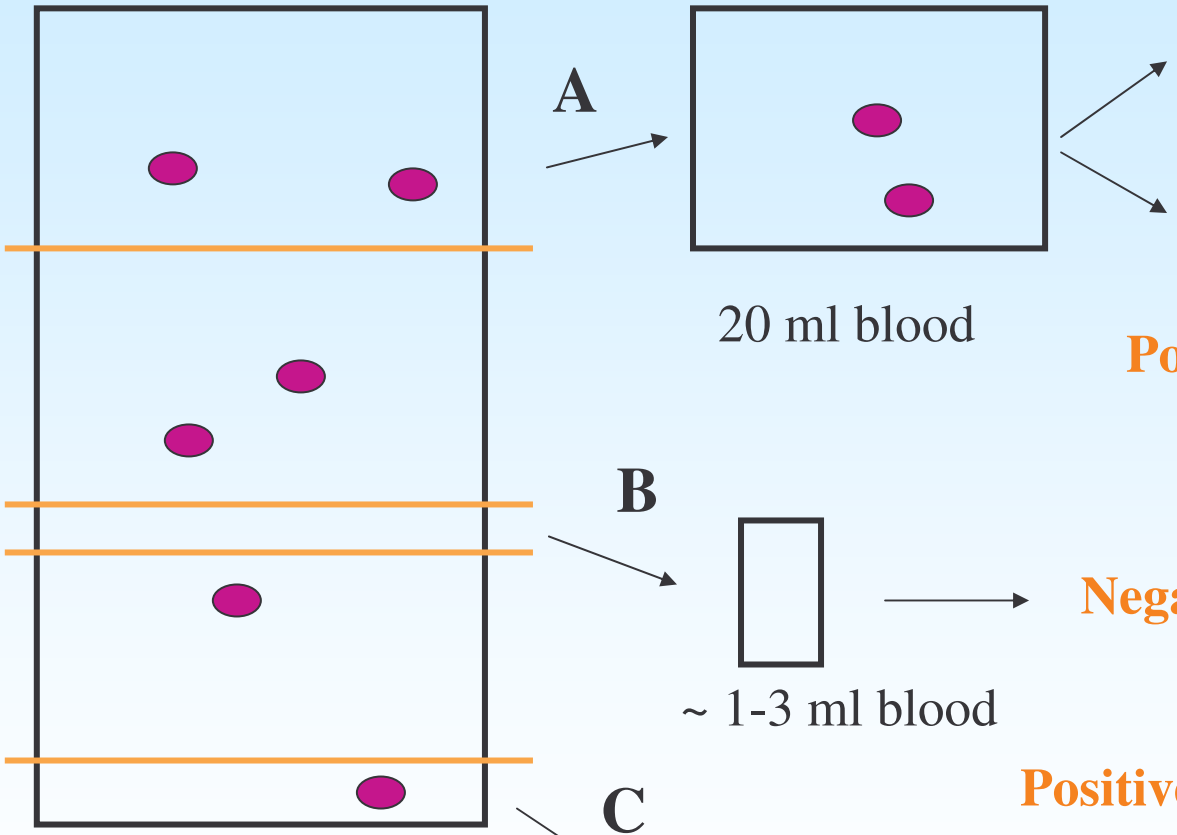
→ 902 serial blood samples processed for BC and PCR

→ 8 proven IC (12 positive BC)



Journal of Medical Microbiology (2009), 58, 1106–1111

Candidemia



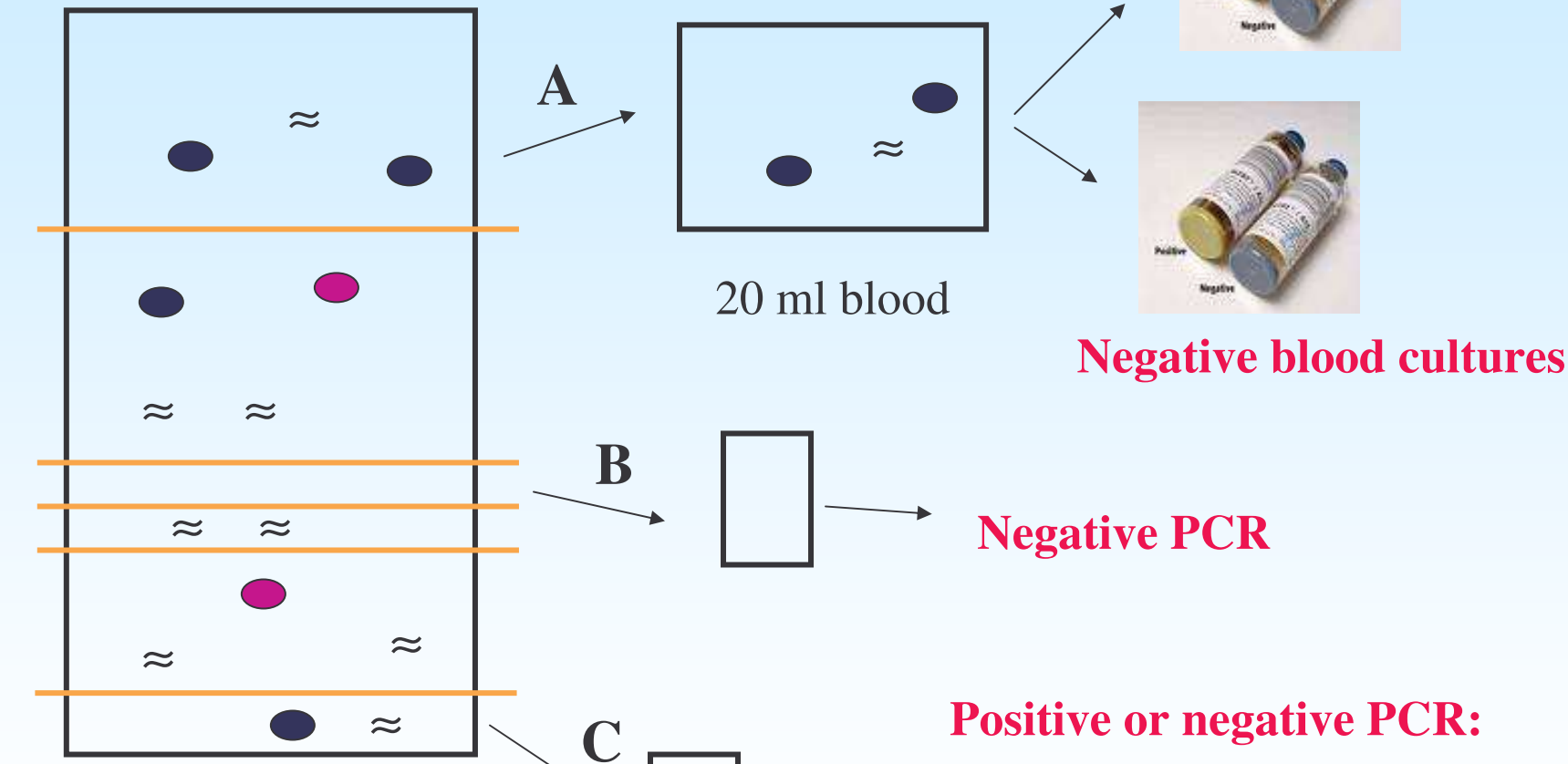
Positive or negative BC

Negative PCR

Positive or negative PCR:

→ serum vs whole blood
→ DNA extraction system
→ PCR limit detection
→ others...

Treated Candidemia



Negative blood cultures

Negative PCR

Positive or negative PCR:

→ serum vs whole blood
→ DNA extraction system
→ PCR limit detection
→ others...

Candida PCR: our experience

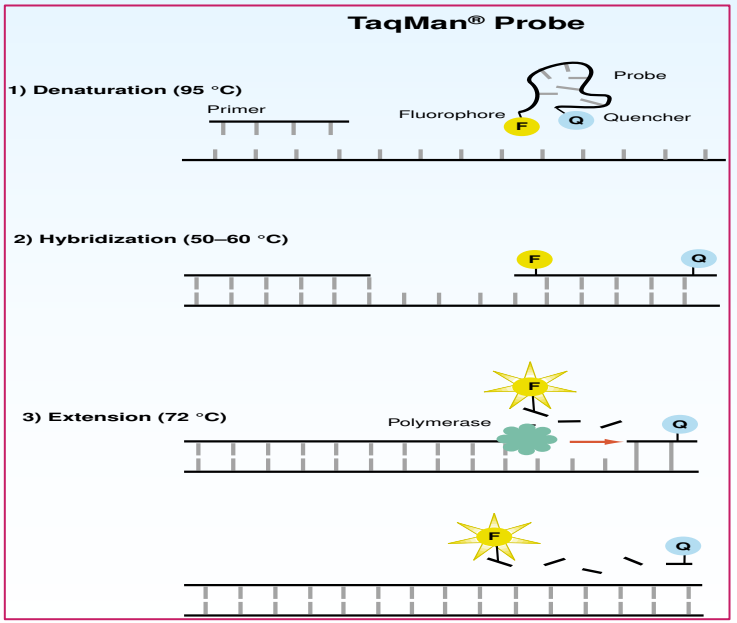
→ Real time PCR (*SmartCycler*, Cepheid)

→ *universal fungal primers* ITS3 i ITS4 (amplification of 18S rDNA)

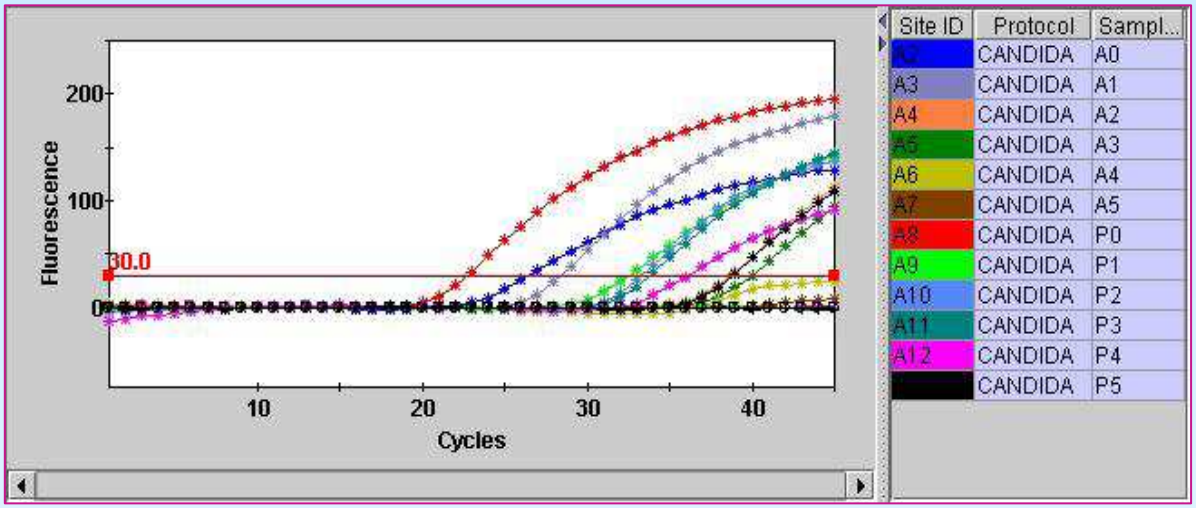
→ *Taqman* probe specific for genus Candida

TABLE 1. Probes used for fluorescence detection of DNA

Probe	Nucleotide sequence (5′ to 3′) and chemistry[a]
All-CAN-TET	5′-TET AG GGC ATG CCT GTT TGA GCG TC(GA) TT-3′-P
CA-FAM	5′-FAM AT TGC TTG CGG CGG TAA CGT CC-3′-P
CT-TET	5′-TET CA AAA CGC TTA TTT TGC TAG TGG CC-3′-P
CP-HEX	5′-HEX GG TAC AAA CTC CAA AAC TTC TTC CA-3′-P
CG-FAM	5′-FAM TA GGT TTT ACC AAC TCG GTG TT GAT-3′-P
CK-TET	5′-TET AG TGG CCC GAG CGA ACT AGA CTT TT-3′-P



JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1999, p. 165–170



Site	Protocol	Sample id	Sample type	FAM/ Result	FAM/ Cycle	TxRed/ Result	TexRed/ Cycle
A2	CANDIDA	albicans	$5x10^5$	POS	26.43	NEG	0.00
A3	CANDIDA	albicans	$5x10^4$	POS	29.42	NEG	0.00
A4	CANDIDA	albicans	$5x10^3$	POS	32.73	NEG	0.00
A5	CANDIDA	albicans	$5x10^2$	POS	35.00	NEG	0.00
A6	CANDIDA	albicans	50	POS	38.92	NEG	0.00
A7	CANDIDA	albicans	5	NEG	0.00	NEG	0.00
A8	CANDIDA	parapsilosis	$5x10^5$	NEG	0.00	POS	22.69
A9	CANDIDA	parapsilosis	$5x10^4$	NEG	0.00	POS	26.53
A10	CANDIDA	parapsilosis	$5x10^3$	NEG	0.00	POS	29.29
A11	CANDIDA	parapsilosis	$5x10^2$	NEG	0.00	POS	32.79
A12	CANDIDA	parapsilosis	50	NEG	0.00	POS	35.19
A13	CANDIDA	parapsilosis	5	NEG	0.00	POS	38.82

Results:

Limits of detection (sensitivity):

C. krusei, C. tropicalis and *C. parapsilosis*: 5 ufc/ml

C. albicans : 50 ufc/ml

C. glabrata: 500 ufc/ml

Especificity**:**

4/150 blood samples from healthy donors resulted positive (E: 99 %)

Candida PCR + conventional culture

165 clinical samples analysed for culture and PCR

143 blood samples

9 CSF

4 articular liquid

4 skin biopsies

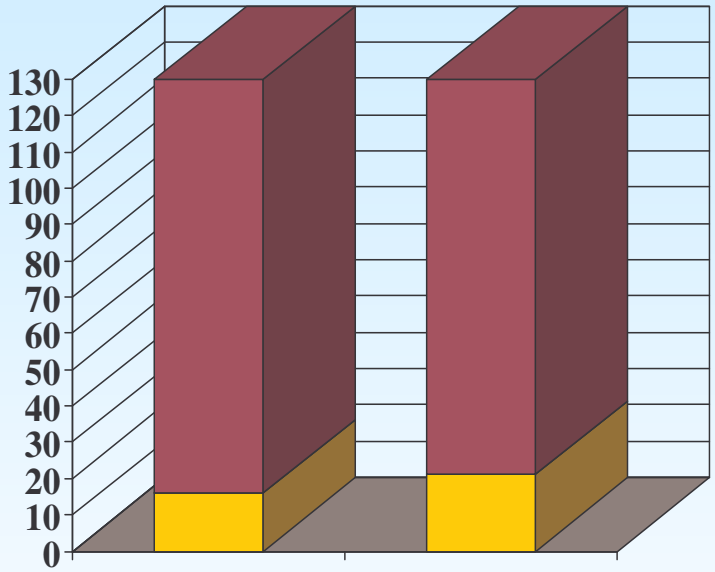
2 pleural líquids

2 BAL

1 lung biopsy

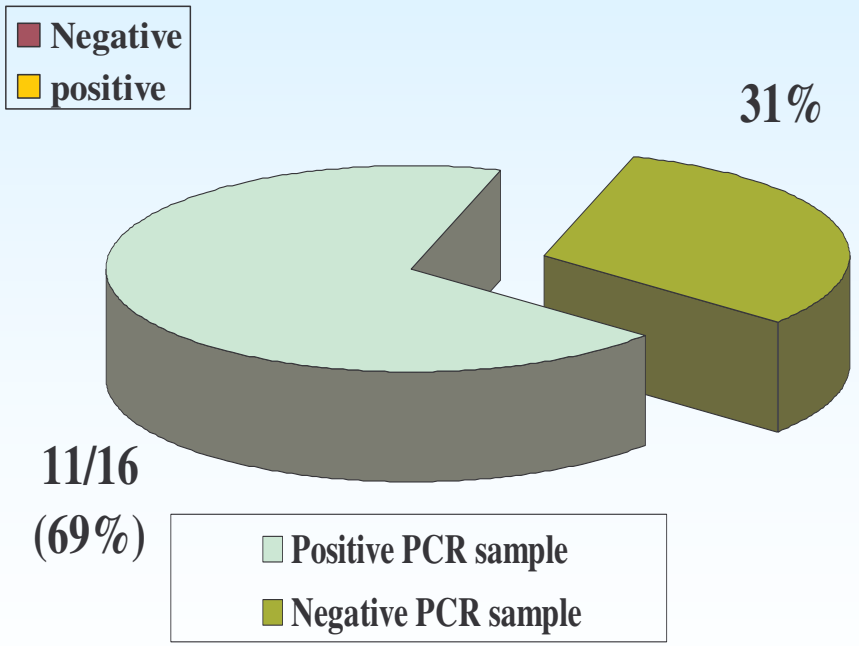
70 Patients at risk of candidiasis (hematology, ICU)

Blood sample results



16/143 (11 %)

21/143 (15.5 %)



16 positive BC samples

Patients:

→ 8 proven candidiasis (Blood or sterile liquid positive for culture). All of them had at least 1 positive sample for PCR (S: 100 %)

→ In 5/8 IC cases, the PCR anticipated the diagnosis 2-3 days

→ 3 cases of “probable candidiasis” (+ PCR /– BC)

Candida PCR project (2011-12)

Evaluation of Magicplex Sepsis real time test (Seegene®) to detect Candida DNA in blood samples belonging to critical patients and patients with febril neutropenia

Hypothesis: The performance of the Candida PCR (*Magicplex™ Sepsis real time test*) in blood samples will increment the diagnostic sensitivity compared to conventional blood culture. For positive BC patients the PCR will provide an earlier diagnosis.

Population to study: critical, oncologic and hematologic patients

To conclude...

→ BC is (still) necessary to detect some cases of IC (samples/patients negative for: mannan, anti-mannan, BG or PCR and positive for BC)

→ Antigens/antibodies detection tests limitations: BG is a panfungal tool and Mn is not present in many species of Candida

→ Next: BC combined with (serial) real time Candida PCR performed in the target population of patients that may benefit the best from these new diagnostic tools


Thank you!
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