3rd European Conference on Infections in Leukemia

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EL:N LeukemiaNet
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EBMT

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ICHS

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1,3- β -D-Glucan for the Diagnosis of Invasive Fungal Infections

F. Lamoth [1], M. Cruciani [2], E. Castagnola [3], O. Lortholary [4], M. Richardson [5], O. Marchetti [1]

1. Infectious Diseases Service, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Switzerland.
2. Center of Preventive Medicine and Infectious Diseases Service, San Bonifacio Hospital, Verona, Italy.
3. Infectious Diseases Unit, Department of Hematology and Oncology, G. Gaslini Children Hospital, University of Genoa, Italy.
4. Service des Maladies Infectieuses et Tropicales, Centre d'Infectiologie Necker-Pasteur, et Centre National de Référence Mycologie et Antifongiques (CNRMA), Institut Pasteur, Paris, France.
5. Regional Mycology Laboratory, Education and Research Centre, University Hospital of South Manchester (Wythenshawe Hospital), United Kingdom.

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BG Assays for Diagnosis of IFI

4 photometric BG assays kits are commercially available for diagnostic use

Kit product	Manufacturer	Availability	Horseshoe crab species	Photometric principle	Manufacturer's Cut-off
Fungitell®	Associates of Cape Cod (ACC), Inc. (USA)	USA (FDA approved), Europe Kit 900 EUR	*Limulus polyphemus*	Chromogenic	60-80 pg/ml > 80 pg/ml
Fungitec G-MK®	Seikagaku Biobusiness (Japan)	Japan only, (collaboration with ACC)	*Tachypleus tridentatus*	Chromogenic	20 pg/ml *
β-Glucan Test®	Wako Pure Chemical Industries, Ltd (Japan)	Japan only	*Tachypleus tridentatus*	Turbidimetric	11 pg/ml *
β-Glucan Test	Maruha Corp. (Japan)	Japan only, collaboration with Wako	*Tachypleus tridentatus*	Chromogenic	11 pg/ml *

* *Tachypleus tridentatus* 3-5x more reactive than *Limulus polyphemus*

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Objective

To Systematically Review the Literature

To Perform a Meta-analysis of the Performance of BG for the Diagnosis of Invasive Fungal Infections

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Methods – Literature Search Criteria

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Keywords / MESH terms:

« beta-glucan » or « glucan »

AND

« fungal infections » or « mycoses » or « candidiasis » or
« candidemia » or « aspergillosis »

Search tools:

- Pubmed
- Embase
- Abstracts presented at international meetings:
  ICAAC, ASM, ECCMID, EBMT, ASCO 2005-2009
- Reviews published in 2005-2009
- English language

Types of Studies

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Study populations

- Patients with hematological malignancies (neutropenia, HSCT)
- Other patients at risk for IFI: ICU, SOT, HIV…

Study designs

- **Case control studies:** IFI vs controls (e.g. healthy blood donors, outpatients, inpatients not at risk of IFI, ….)
- **Cohort studies:** prospective screening of BG (consecutive sampling method) in a homogenous patients population at risk for IFI (e.g. hematological, ICU, SOT…)

Assessment of the methodological quality of the studies

- Recommendations of the Standards for Reporting of Diagnostic Accuracy (SRDA) using the QUADAS tool (14 items).

Methods – Selection Criteria

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Inclusion Criteria:

- Clinical studies (pro-/retro-spective)
- Population at risk for IFI (HEM, ICU, SOT…)
- BG measurements in IFI (blood, other samples)

Exclusion Criteria:

- Non commercially available BG assay (other than: Fungitell, Fungitec-G, Wako-Maruha)
- Insufficient sample size (<5 IFI or <30 pts)
- Inappropriate reference standard for IFI (EORTC-MSG criteria *)
- Lack of data for assessment of EORTC-MSG criteria (studies performed before 2002)
- No BG measurement in non-IFI cases (no data on specificity)

 * Ascioglu et al. Clin Infect Dis 2002

Study Selection

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- 861 studies were screened
- 29 Studies met inclusion criteria
- 6 were excluded:
  - 2 inappropriate standard reference for IFI
  - 2 lack of data for calculation of performance
  - 1 non validated BG test
  - 1 no English language
- 23 were selected for analysis:
  - 12 (+1) case control studies
  - 10 (+1) cohort studies

Case Control Studies – BG Performance for Diagnosis of Proven/Probable IFI

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Fungitell (Associates of Cape Cod)					
Cut-off	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Efficiency**
60 pg/ml *	70	87	84	75	NA
80 pg/ml **	47 - 93	71 - 100	52 - 88	77 - 98	74 - 80
Fungitec-G (Seikagaku)					
Cut-off	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Efficiency**
20 pg/ml ***	58 – 100	100	59 – 100	40 – 100	79 - 100
60 pg/ml ****	76 – 85	95 – 100	70 – 100	75 – 98	86 - 91

* Ostrosky-Zeichner
** Hachem, Persat, Alam, Pickering, Del Bono
*** Obayashi (1995), Hossain, Miyazaki, Kondori
**** Obayashi (2008), Kohno, Mitsutake

Efficiency: (true positives + true negatives) / total number of tests

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Case Control Studies – Comments

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- Major limitations in study design:
  - Heterogeneity of controls and IFI patients (HEM, ICU…)
  - Lack of data on sampling time (vs. diagnosis of IFI ?)
  - Most retrospective analyses: bias ?
- Limitations related to types of BG assays:
  - Different cut-offs used
  - Many studies performed in the 1990s (tests' evolution with multiple modifications in BG assay technique ?)
  - No studies with BG assays Wako / Maruha
- Variable performance
  - Sensitivity: 50-90%
  - Specificity: 70-100%
  - Efficiency: 75-90%

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Cohort Studies – BG Performance

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Proven/probable IFI (Proven/probable/possible IFI)

Fungitell (Associates of Cape Cod)						
Study	**Cut-off tested**	**Optimal cut-off ***	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Efficiency**
Ellis 2008 J Med Microbiol	60-100 pg/ml (1 or 2 values)	100 pg/ml (2 values)	- (82)	- (83)	- (82)	- (83)	- (83)
Koo 2006 ICAAC (abstract)	80 pg/ml (1 value)	80 pg/ml (1 value)	71 (-)	86 (-)	- (-)	- (-)	- (-)
Pazos 2006 Rev Ibero Micol	120 pg/ml (1 value)	120 pg/ml (1 value)	83 (-)	90 (-)	63 (-)	96 (-)	89 (-)
Pazos 2005 J Clin Microbiol	120 pg/ml (1 value)	120 pg/ml (1 value)	88 (73)	90 (90)	70 (73)	96 (90)	89 (85)
Odabasi 2004 Clin Infect Dis	60 pg/ml (1,2,3 values)	60 pg/ml (3 values)	60 (28)	100 (100)	100 (100)	97 (86)	97 (87)
Presterl 2009 Int J Infect Dis	40 pg/ml (1 value)	40 pg/ml (single)	50 (-)	76 (-)	46 (-)	79 (-)	68 (-)
Del Bono 2009 ICAAC (abstract)	80 pg/ml (1 value)	80 pg/ml (1 value)	100 (100)	67 (67)	75 (87)	100 (100)	83 (90)

Hemato-oncological population with exception of : Presterl 2009 (ICU), Del Bono 2009 (ICU).

* Cut-off with best efficiency

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Cohort Studies – BG Performance

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Proven/probable IFI (Proven/probable/possible IFI)

Fungitec-G (Seikagaku)						
Study	**Cut-off tested**	**Optimal cut-off ***	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Efficiency**
Kami 2000 Hematologica	20 pg/ml (1 value)	20 pg/ml (1 value)	63 (60)	76 (76)	19 (29)	96 (92)	75 (74)
Akamatsu 2007 Infection	40 pg/ml (1 value)	40 pg/ml (1 value)	63 (-)	83 (-)	32 (-)	95 (-)	81 (-)
BG (Wako / Maruha)						
Kawazu 2004 J Clin Microbiol	2 - 11 pg/ml (1 or 2 values)	11 pg/ml (2 values)	45 (25)	99 (99)	83 (71)	95 87)	95 (87)
Senn 2008 Clin Infect Dis	3 - 11 pg/ml (1 or 2 values)	7 pg/ml (2 values)	63 (37)	96 (96)	79 (81)	91 (74)	89 (75)

Hemato-oncological population with exception of Akamatsu 2007 (solid-organ transplant).

* Cut-off with best efficiency

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Cohort Studies – BG Performance

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Proven+probable invasive candidiasis (IC) /
Proven+probable invasive aspergillosis (IA)

BG Assay	Cut-off	Sensitivity	Specificity	PPV	NPV	Efficiency
Fungitell	60-120 pg/ml (1-3 values)	67 – 83 / 25 – 88	90 – 100 / 90 – 100	63 – 100 / 70 – 100	96 – 98 / 96 – 99	89 – 98 / 89 – 99
Fungitec-G	20-40 pg/ml (1 value)	50 / 63 – 100	83 / 76 – 83	21 / 16 – 19	95 / 96 – 100	81 / 75 – 84
Wako / Maruha	7-11 pg/ml (2 values)	59 / 45 – 60	96 / 96 – 99	67 / 64 – 83	94 / 95	91 / 91 – 95

Limitations

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- Few comparable studies (stratified according to type of BG assay).
- Few - missing data on:
  - Timing/Frequency of BG assay for screening
  - Neutropenia vs. non-neutropenia / AL vs. allo-HSCT
  - ICU, SOT, children, ….
  - Site of IFI: fungemia vs. deep-seated infection
  - Influence of antifungal prophylaxis-therapy on BG
  - Impact of 2008 EORTC/MSG definitions on performance ? BG included in diagnostic criteria !
  - IFI other than IA / IC (zygomycosis, …); *P. jirovecii* in non-HIV
  - Utility in BAL, other biological fluids
  - Cost effectiveness.

Cohort Studies – Comments

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- For each BG assay 1-2 high quality cohort studies with significant sample size in hematological patients :
  - Fungitell: Odabasi (CID 2004), Ellis (JMM 2008).
  - Fungitec-G: Kami (Hematologica 2000).
  - BG (Wako / Maruha): Kawazu (JCM 2004), Senn (CID 2008).
- Variable performance of BG for diagnosis of proven/probable IFI:
  - Sensitivity: 45 – 70% / Specificity: 75 – 100%
  - PPV: 80 – 100% / NPV: 90 – 95%
  - Efficiency: 75 – 95%.
- Similar performance of BG for the diagnosis of IA / IC.
- Global performance (efficiency) similar comparing one single and 2 or more consecutive positive values.

Comparison of BG with Other Diagnostic Tests

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BG test Cut-off	Comparative test Cut-off	Sensitivity	Specificity	PPV	NPV	Efficiency
Beta-glucan / galactomannan						
Fungitell (3) [1] 80-120 pg/ml	Platelia ODI=0.5-1.5	67 – 88 / 38 – 88	73 – 90 / 56 – 100	64 – 88 / 47 – 100	72 – 96 / 61 – 96	71 – 89 / 56 – 89
Wako/Marhua (2) [2] 7-11 pg/ml (2x)	Platelia ODI=1 (2x)	45 – 60 / 36 – 64	96 – 99 / 98 – 100	64 – 83 / 70 – 100	95 / 91 – 97	91 – 95 / 94 – 95
Beta-glucan / mannan and/or anti-mannan						
Fungitell (2) [3] 80 pg/ml	M: 0.5 ng/ml or AM: 10 AU/ml	52 – 87 / 48 – 81	70 – 100 / 73 – 100	100 / 100	55 / 76	70 / 88
Wako/Marhua (1) [4] 7 pg/ml (2x)	M: 0.5 ng/ml or AM: 10 AU/ml	60 / 69	96 / 95	64 / 64	95 / 96	91 / 92

[1] Pazos 2005, Persat 2008, Hachem 2009
[2] Kawazu 2004, Senn 2008
[3] Alam 2007, Persat 2008
[4] Senn 2008

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Conclusions

- Beta-glucan is a screening test that may identify patients with invasive fungal infections, such as invasive aspergillosis and invasive candidiasis.
- Available data suggest that beta-glucan is a reliable test to estimate the diagnostic accuracy for these invasive fungal infections in adults only.
- A frequency of 2 tests per week which was performed in most studies seems an appropriate screening strategy.
- Results of the beta-glucan assay may complement clinical, radiological and laboratory criteria for the diagnosis of IFI.
- The threshold for positive results depends on the test which is used. Evidence from the available data suggest the following cut-off:
  – Fungitell: between 60 and 80 pg/ml.
  – Wako / Maruha: between 7 and 11 pg/ml
  – Fungitec-G: 20 pg/ml.
- The criteria of two consecutive specimens to define the test as positive increases the specificity but decreases the sensitivity.

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Recommendations

- Screening of BG in plasma for the diagnosis of IFI is recommended in high-risk hematological patients (prolonged neutropenia after induction/consolidation chemotherapy for AL or allogeneic HSCT).

  Strength for recommendation: moderate evidence to support recommendation for use.

  Quality of evidence: evidence from well-designed cohort studies.

  IDSA-CDC grading: BII

Requirements for future studies

Screening strategy (frequency of samplings) and optimal cut-offs should be assessed in further analyses.

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Warnings

False positive results may be associated with:
- Concomitant antimicrobial therapy (beta-lactams)
- Bacteremias
- Hemodialysis patients (cellulose filters)
- Patients receiving coagulation factors / albumin / immunoglobulins
- Hemolyzed serum specimens
- Contaminated specimens (gauze for desinfection at the bedside / environmental dusts-organic wastes in the lab)

False negative results may be associated with:
- Zygomycosis, cryptococcosis, other fungal infections
- Antifungal therapy (?)

Attention should be paid to the technical complexity of the assay and the cost implications.

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IDSA-United States Public Health Service Grading System for Ranking Recommendations

Quality of evidence	Strength of recommendation
I Evidence from $\geq$ 1 properly randomized, controlled trial	A Good evidence to support a recommendation for use
II Evidence from $\geq$ well-designed clinical trial, without randomization; from cohort or case-controlled analytic studies (preferably from >1 center); from multiple time-series; or from dramatic results from uncontrolled experiments	B Moderate evidence to support a recommendation for use
III Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees	C Poor evidence to support a recommendation

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