Biomarkers in fungal diagnosis: diagnostic interpretation

Dr Ram Gopalakrishnan
Tests available in India

- Procalcitonin (PCT)
- Aspergillus galactomannan (GM)
- Beta d glucan (BDG)
PROCALCITONIN
ProCT – the molecular basis for the increase in inflammation and infection

- Mature CT is produced mostly in neuro-endocrine C-cells of the thyroid. In the absence of infection, the extrathyroidal transcription of the CALC-I gene is suppressed and is restricted to a selective expression in neuro-endocrine cells found mainly in the thyroid and lung.
- In these neuroendocrine cells, the mature hormone is processed and stored in secretory granules.
- Interestingly, a microbial infection induces an ubiquitous increase of CALC-I gene-expression and a constitutive release of ProCT from all parenchymal tissues and differentiated cell types throughout the body.

Procalcitonin in bacterial infections – hype, hope, more or less?

SWISS MED WKLY 2005; 135: 451–460
Under septic circumstances, the entire body could be viewed as being an endocrine gland. Indeed, the transcriptional expression of CT-mRNA is more uniformly up-regulated in sepsis than are the mRNAs of the classical cytokines (e.g., tumour necrosis factor (TNF)-α and interleukin (IL)-6).
Procalcitonin

- Levels correlate with severity of sepsis
- Not elevated in viral infections or fungal infections
- IFI triggers a different pattern cytokine response from those induced by bacterial sepsis.
- May go up in non-infectious inflammatory states

- Meta-analysis of studies for detection of sepsis:
  - mean sensitivity of 0.77 and specificity of 0.79.
  - must be interpreted carefully in the context of medical history, physical examination, and microbiological assessment.

- Best studied in regard to decision to stop antibiotics

- Strategy of escalation (ie, broadening) of antimicrobial spectrum in critically ill patients based on PCT levels did not work

(Lancet 2010;375:463) (Crit Care Med 2012;40;2034)
(Crit Care Med 2011; 39:2048–58)
(Lancet Infect Diseases, Early Online Publication, 1 February 2013)
Procalcitonin-guided diagnosis and antibiotic stewardship revisited

Ramon Sager¹², Alexander Kutz¹², Beat Mueller¹² and Philipp Schuetz¹²*¹

Abstract

Several controlled clinical studies have evaluated the potential of the infection biomarker procalcitonin (PCT) to improve the diagnostic work-up of patients with bacterial infections and its influence on decisions regarding antibiotic therapy. Most research has focused on lower respiratory tract infections and critically ill sepsis patients. A clinical utility for PCT has also been found for patients with urinary tract infections, postoperative infections, meningitis, and patients with acute heart failure with possible superinfection (i.e., pneumonia). In these indications, PCT levels measured on hospital admission were found to substantially reduce the initiation of antibiotic treatment in low-risk situations (i.e., bronchitis, chronic obstructive pulmonary disease exacerbation). For more severe infections (i.e., pneumonia, sepsis), antibiotic stewardship by monitoring of PCT kinetics resulted in shorter antibiotic treatment durations with early cessation of therapy. Importantly, these strategies appear to be safe without increasing the risk for mortality, recurrent infections, or treatment failures. PCT kinetics also proved to have prognostic value correlating with disease severity (i.e., pancreatitis, abdominal infection) and resolution of illness (i.e., sepsis). Although promising findings have been published in these different types of infections, there are a number of limitations regarding PCT, including suboptimal sensitivity and/or specificity, which makes a careful interpretation of PCT in the clinical context mandatory. This narrative review aims to update clinicians on the strengths and limitations of PCT for patient management, focusing on research conducted within the last 4 years.
Fig. 1 Summary of evidence regarding procalcitonin (PCT) for diagnosis and antibiotic stewardship in organ-related infections. While for some infections, intervention studies have investigated benefit and harm of using PCT for diagnosis and antibiotic stewardship (left side), for other infections only results from diagnostic (observation) studies are available (right side). +: moderate evidence in favor of PCT; ++: good evidence in favor of PCT; +++: strong evidence in favor of PCT; - no evidence in favor of PCT.
**SUSPECTED SEPSIS IN CRITICALLY ILL PATIENT**

- Not severely immunocompromised (other than corticosteroids)
- Not on antibiotics for chronic bacterial infection (e.g. endocarditis, osteomyelitis)

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Obtain baseline PCT but do not delay antibiotics

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Repeat PCT daily

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Stop antibiotics when PCT ≤ 0.5 pg/L or decrease by ≥ 80% from peak (and patient clinically improving)
Procalcitonin for the diagnosis of invasive candidiasis: what is the evidence?

Santi Maurizio Raineri, Andrea Cortegiani, Filippo Vitale, Pasquale Iozzo and Antonino Giarratano

- significantly lower PCT level in patients with candidemia (median 0.65 ng/ml) compared to those with bacteremia (median 9.75 ng/ml)
- correlation between a low PCT level (< 2 ng/ml) and Candida infection
- high NPV of PCT for Candida isolation
• Procalcitonin values were found to be significantly lower in patients with candidemia (0.73; IQR 0.26-1.85 ng/mL) than in those with bacteremia (4.48; IQR 1.10-18.26 ng/mL).

• At ROC curve analysis, values of PCT greater than 2.5 ng/mL had a negative predictive value (NPV) of 98.3% with an AUC of 0.76 (0.68-0.84 95% CI) for the identification of Candida spp. from blood cultures.

• At multivariate analysis, a PCT value <2.5 ng/mL showed an odds ratio of 8.57 (95% CI 3.09-23.70; p < 0.0001) for candidemia.

• They concluded that in septic patients at risk of Candida infection, a PCT value lower than 2.5 ng/mL should raise the suspicion of candidemia.
• 292 septic patients with positive blood culture
• serum Procalcitonin values in Gram-negative, Gram-positive, and fungal sepsis were 7.47 (interquartile range [IQR]: 1.09-41.26) ng/mL, 0.48 (IQR: 0.15-2.16) ng/mL, and 0.60 (IQR: 0.14-2.06) ng/mL, respectively ($P< 0.001$).
• An optimal cut-off value of 3.11 ng/mL for PCT was found to be useful in discriminating Gram-negative sepsis from fungal sepsis, which led to a sensitivity of 63.9% and specificity of 93.3%.
• median PCT value in Gram-negative (13.8 ng/mL, interquartile range (IQR) 3.4-44.1) bacteremias was significantly higher than in Gram-positive (2.1 ng/mL, IQR 0.6-7.6) or fungal (0.5 ng/mL, IQR 0.4-1) infections (P < 0.0001).

• Receiver operating characteristic analysis showed AUC of 0.944 (95% CI 0.919-0.969, P < 0.0001) in discriminating Gram-negatives from fungi at the best cut-off of 1.6 ng/mL.
Four studies compared IFI to bacterial sepsis:
– pooled positive likelihood ratio 4.65 (95% confidence interval [CI], 2.46–8.79)
– negative likelihood ratio 0.15 (95% CI, 0.05–0.41)
Septic shock with clinical possibility of fungal infection

- Low PCT: consider fungal infection
- High PCT: fungal infection less likely

- It is important to realize that PCT is of little to moderate diagnostic value for differentiating fungal infection from other non-infections conditions
- Gram positive infections also have lower PCTs
ASPERGILLUS GALACTOMANNAN
Aspergillus GM

- Galactomannan is a cell wall component of *Aspergillus* spp. and of *Penicillium* spp
- Also present in the cell wall of *Penicillium*, *Fusarium*, *Alternaria*, *Acremonium*, and *Histoplasma capsulatum*
- It is excreted by the fungus during the growth phase.
- Galactomannan reflects live dividing fungi so can't be positive in colonization, unlike PCR
- Amount of galactomannan is proportional to the fungal load in the tissue and that the amount of galactomannan has a prognostic value
Step 1
Monoclonal antibody: mAb EB-2
Antibody source: mouse
Microplate coated with mAb EB-A2 (capture Ab)
Capture antibody

Step 2
Antigen

Step 3
Detecting antibody
Enzyme

Step 4
Yellow artificial substrate
Optical Reader

Fig. 2 Schematic representation of *Aspergillus* galactomannan ELISA immunoassay procedure. Step 1: Microplate wells are coated with murine monoclonal antibody (mAb) EB-A2 (capture antibody). Step 2: Treated serum or bronchoalveolar lavage sample is added to the wells. Step 3: Labeled anti-mAb EB-A2 antibody (detecting antibody) is added to the wells. In the presence of the antigen (EB-A2) a “mAb–antigen–labeled anti-mAb complex” is formed. Step 4: Substrate solution is added to the wells and the presence of “mAb-antigen–labeled anti-mAb complex” is revealed by forming a blue color. An optical reader is then used to calculate the optical density index (i.e., galactomannan test result).
Invasive aspergillosis primarily occurs in patients who have specific risk factors, such as
- prolonged neutropenia,
- history of allogeneic hematopoietic cell or solid organ transplantation,
- use of high-dose corticosteroids
- inherited severe immunodeficiency.

Dense, well-circumscribed nodular lesion(s) on CT scan, with or without surrounding hazy infiltrate (halo sign) and cavitary lesions, are characteristic but not specific for invasive pulmonary aspergillosis.
<table>
<thead>
<tr>
<th>Pre-test probability</th>
<th>BDG / GM testing (serum)</th>
<th>Post-test probability</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic cancer / Neutropenia / HSCT</td>
<td>Lung nodules with halo or air-crescent sign</td>
<td>High (++)</td>
<td>+++</td>
</tr>
<tr>
<td>Solid-organ transplantation</td>
<td>Aspecific lung nodules</td>
<td>Moderate (+)</td>
<td>+ to ++</td>
</tr>
<tr>
<td>Auto-immune diseases / Solid cancer / corticoid therapy</td>
<td>Aspecific lung infiltrate</td>
<td>Low (+/-)</td>
<td>+ / -</td>
</tr>
</tbody>
</table>

Test uninterpretable. Sensitivity and specificity are both low (or unknown) in this setting.
Serum Aspergillus galactomannan

• Antigen test that reflects Aspergillus burden
• Results of the ELISA are given as an optical density index (ODI), which is the ratio of the optical density of (usually) 1 ng/ml galactomannan versus the optical density of the sample.
• The mean sensitivity of the galactomannan ELISA (Cochrane Database of Systematic Reviews 2015)
  – at a cut-off of 0.5 optical density index (ODI) was 78% (70% to 85%) and the specificity was 85% (78% to 91%).
  – At a cut-off value of 1.0 ODI, sensitivity was 71% (63% to 78%) and specificity was 90% (86% to 93%).
  – At a cut-off value of 1.5 ODI, sensitivity was 63% (49% to 77%) and specificity was 93% (89% to 97%).
• Two values of >0.5 or one value of >1.0
• Pay attention to the exact value: higher is more specific
• The *Aspergillus galactomannan enzyme immunoassay detects* polysaccharides that are present in the cell wall of *Aspergillus species* and that can be found in serum and bronchoalveolar lavage fluid during invasive infection.

• The role of the galactomannan assay in the diagnosis of invasive aspergillosis has been studied most often in *neutropenic patients* and *allogeneic hematopoietic cell transplant recipients*.

• In these patient groups, the reported sensitivity of the assay
  – in serum is 70%-82% and specificity is 81%-92%
  – in bronchoalveolar lavage fluid, sensitivity is 73%-100% and specificity is 68%-92%.


Clinical role

• Very sensitive and specific in the neutropenic patient with invasive Aspergillosis
• Pre-emptive strategy: Routinely do twice weekly for high risk neutropenic host not receiving antifungal prophylaxis
• Not recommended for
  – asymptomatic neutropenic patients undergoing effective prophylaxis
  – screening in SOT recipients
  – patients with CGD
• Useful in assessing response after therapy  (Clin Infect Dis 2011;53:671)
• BAL GM had a sensitivity of 90% and a specificity of 94.0%, when cut-off of 0.5 -1.0 was used
• sensitivity is 91% and specificity is 88% (Clin Infect Dis 2009;49;1688)
• GM levels were significantly increased in those receiving antibacterial therapy at the time of bronchoscopy ($P=0.002$)
• Can use even in patients on mold-active antifungal therapy or prophylaxis, unlike serum
• A meta-analysis in 2010 reported a sensitivity of 90% and a specificity of 94%
• In order to enhance the specificity of the test, the FDA changed the recommended cut-off for positivity in BAL in the United States from 0.5 to 1.0 ODI.
Solid organ transplant

• In solid organ transplant recipients
  – sensitivity is 21%-86% and specificity is 80%-89% in serum
  – in broncholveolar lavage fluid, sensitivity is 60%-90% and specificity is 90%-96%.
  


• The sensitivity of the assay is higher in bronchoalveolar lavage fluid than in serum, especially in lung transplant recipients.

False positives seen with galactomannan

- Pasta, rice, canned vegetables
- Geotrichum, histoplasma, fusarium infections
- Electrolyte solutions containing sodium gluconate
- Bifidobacterium bifidum therapy
- IVIG therapy
- Severe mucositis
- Severe gastrointestinal GVHD
- Blood products collected in certain commercially available infusion bags
- Multiple myeloma (IgG type)
- Flavored ice pops or frozen desserts containing sodium gluconate.
Antibiotics can cause false positive GM

- **piperacillin-tazobactam** reported in the past, but manufacturing changes have eliminated this problem
- Ampicillin, amox-clav, phenoxyimethypenicillin
- At the cut-off index of ≥0.5, false-positive serum results were found in patients who received amoxicillin–clavulanate, piperacillin–tazobactam, cefepime, and cefoperazone–sulbactam (26.7%, 58.3%, 14.3%, and 66.7%, respectively)
  - these semisynthetic drugs are derived from natural compounds produced by molds of the genus *Penicillium* that contain in the cell wall galactofuran-bearing molecules

BETA D GLUCAN ASSAY
• Beta D glucan assay is a US FDA approved quantitative assay used to aid in the detection of invasive fungal infections.
• The BG component is composed mainly of glucose polymers linked via β-1,3-glycosidic bonds, forming the BG backbone of the fungal cell wall.
• As the fungus grows and divides, this cell wall is continuously re-modelled and some BG is released as soluble forms.
• The basis of the beta D glucan assay relies on BG’s ability to activate the limulus amebocyte lysate clotting cascade present in the blood of Limulus polyphemus.

• The resultant clotting can be measured via spectrophotometry, yielding an indirect BG concentration in picograms per millimeter (pg/mL).
Application of the 1,3-β-D-Glucan (Fungitell) Assay in the Diagnosis of Invasive Fungal Infections. Tran T, Beal SG
• A BG value of < 60 pg/mL is considered a negative result
• 60 to 79 pg/mL is an indeterminate result
• 80 pg/mL or more is a positive result.

• Associates of Cape Cod. Assay for (13)-b-D-glucan in serum: Fungitell—
  Fungitell_multilang_pisheets/Fungitell%20Insert%20EN.pdf. Updated
Fungi Detected By BDG Assay

- Candida spp
- Aspergillus spp
- Fusarium spp
- Pneumocystis jiroveci
- Coccidioides immitis
- Histoplasma capsulatum
- Blastomyces dermatidis

Not Detected By BDG Assay

- Cryptococcus species

- Zygomycetes (Lichtheimia corymbifera, Mucor spp, Rhizopus spp)

Pan fungal (1,3- Beta-D mannann) antigen

- Think of as “fungal endotoxin”, very non-specific
- Cut-off is 80 pg/ml
- Sensitivity of 76% and specificity of 85% in meta-analysis (Clin Infect Dis 2011;52:750)

-Good negative predictive value

β-D-glucan levels ≥80 pg/mL predicted intra-abdominal candidiasis and did so a median of 5 days before culture positivity (Am J Respir Crit Care Med 2013 Nov 1; 188:1048).

• Yield in Candidemia (CID 2012)
  • Blood culture + PCR: 98%
  • Blood culture + Beta d glucan: 79%

Useful both in neutropenic patients and ICU setting- sensitivity 71% and specificity 81% (Clin Infect Dis 2009;49:1650)
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 Funata²

Departments of ¹Laboratory Medicine and ²Pathology, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan
Salient features

- This study of 456 autopsy cases

<table>
<thead>
<tr>
<th>Feature</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity of blood culture</td>
<td>8.3%</td>
</tr>
<tr>
<td>Sensitivity of BDG assay</td>
<td>78%</td>
</tr>
<tr>
<td>Specificity of BDG assay</td>
<td>98.4</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>86%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>98%</td>
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</tbody>
</table>
β-d-Glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis

Drosos E. Karageorgopoulos,¹,² Evridiki K. Vouloumanou,¹ Fotinie Ntziora,¹,² Argyris Michalopoulos,¹,³ Petros I. Rafailidis,¹,⁴ and Matthew E. Falagas¹,⁴,⁵

¹Alfa Institute of Biomedical Sciences; ²Department of Medicine, Laikon General Hospital, and ³Intensive Care Unit and ⁴Department of Medicine, Henry Dunant Hospital, Athens, Greece; and ⁵Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts
Salient features

- Meta-analysis of 2979 patients in 16 studies
- Sensitivity of 76.8%.
- Specificity of 85.3%
- Positive predictive value of 85%
- **Negative predictive value of 99%**
• **Prognostic value:**
  – Severe sepsis (91% versus 28%) and mortality (36% versus 6%) were significantly higher in patients with BG result of \textit{400 pg/mL} or more compared to those with a BG result of less than 400 pg/mL.

• **Therapeutic value:**
  – BG levels decreased in patients responding to therapy but continued to rise or remain elevated in patients who did not respond.
β-d-Glucan as a Diagnostic Adjunct for Invasive Fungal Infections: Validation, Cutoff Development, and Performance in Patients with Acute Myelogenous Leukemia and Myelodysplastic Syndrome

Zekaver Odabasi,1 Gloria Mattiuzzi,2 Elihu Estey,2 Hagop Kantarjian,2 Fumihiro Saeki,3 Richard J. Ridge,3 Paul A. Ketchum,3 Malcolm A. Finkelman,3 John H. Rex,1,a and Luis Ostrosky-Zeichner1

1Laboratory of Medical Mycology, University of Texas–Houston Medical School, and 2The University of Texas M.D. Anderson Cancer Center, Houston, Texas; and 3Associates of Cape Cod, Falmouth, Massachusetts
Salient features

• 283 patients with AML or MDS on antifungal prophylaxis
• Serial BG testing from these patients and found that BG became positive a median of 10 days before the clinical diagnosis of proven or probable invasive fungal infection was determined.
• All patients were receiving antifungal prophylaxis, this intervention did not seem to affect the performance of the test.
• BDG also recommended by IDSA for Aspergillus diagnosis
β-Glucan Antigenemia Anticipates Diagnosis of Blood Culture–Negative Intraabdominal Candidiasis

Frederic Tissot¹, Frederic Lamothe¹, Philippe M. Hauser², Christina Orasch¹,³, Ursula Flückiger³, Martin Siegemund⁴, Stefan Zimmerli⁵, Thierry Calandra¹, Jacques Bille², Philippe Eggimann⁶*, Oscar Marchetti¹*, and the Fungal Infection Network of Switzerland (FUGINOS)

¹Infectious Diseases Service, Department of Medicine, ²Institute of Microbiology, and ⁶Adult Intensive Care Service, Lausanne University Hospital, Lausanne, Switzerland; ³Division of Infectious Diseases and Hospital Epidemiology and ⁴Intensive Care Service, Basel University Hospital, Basel, Switzerland; and ⁵Institute for Infectious Diseases, University of Bern, Bern, Switzerland
Salient features

- BG testing was superior to Candida score and colonization index, and BG became positive a median of 5 days prior to culture-based diagnosis of intra-abdominal candidiasis.
Improved diagnostics will identify cases of invasive candidiasis, in particular deep-seated candidiasis, that are currently missed by cultures.
Clinical findings suggested an association between reduced invasive candidiasis incidence in ICUs and BDG guided preemptive antifungal therapy.

In base-case analysis, the surveillance group was more costly (1387 USD versus 664 USD) (1 USD = 7.8 HKD), with lower candidiasis-associated mortality rate (0.653 versus 1.426 per 100 ICU admissions) and QALY loss (0.116 versus 0.254) than the standard care group.

BDG guided antifungal therapy was found to be highly cost effective to reduce candidiasis associated mortality rates and save quality adjusted life year (QALY) in ICU settings.
Accuracy of $\beta$-d-glucan for the diagnosis of Pneumocystis jirovecii pneumonia: a meta-analysis

D. E. Karageorgopoulos$^{1,2,3}$, J.-M. Qu$^4$, I. P. Korbila$^1$, Y.-G. Zhu$^4$, V. A. Vasileiou$^5$ and M. E. Falagas$^{1,6,7}$

1) Alfa Institute of Biomedical Sciences (AIBS), 2) Department of Medicine, Hygeia Hospital, 3) Hellenic Center for Disease Control and Prevention, Marousi, Athens, Greece, 4) Department of Pulmonary Medicine, Huadong Hospital, School of Medicine, Fudan University, Shanghai, China, 5) School of Applied Mathematical and Physical Sciences, National Technical University of Athens, 6) Department of Medicine, Henry Dunant Hospital, Athens, Greece and 7) Department of Medicine, Tufts University School of Medicine, Boston, MA, USA
• BDG data were analysed for 357 PCP cases and 1723 controls.
• Sensitivity 94.8%
• Specificity 86.3%.
• Positive likelihood ratios 6.9
• Negative likelihood ratios 0.06.
• So a positive BDG with compatible clinical and radiological findings result can be a strong indicator for the presence of PCP.
Beta Glucan Assay in Children

Quantification of 1,3-β-d-Glucan Levels in Children: Preliminary Data for Diagnostic Use of the β-Glucan Assay in a Pediatric Setting

P. Brian Smith,1,2 Daniel K. Benjamin, Jr.,1,2 Barbara D. Alexander,3 Melissa D. Johnson,3 Malcolm A. Finkelman,4 and William J. Steinbach1,5*

Department of Pediatrics, Duke University Medical Center, Durham, North Carolina1; Duke University Clinical Research Institute, Durham, North Carolina2; Department of Medicine, Duke University Medical Center, Durham, North Carolina3; Associates of Cape Cod, Inc., Falmouth, Massachusetts4; and Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina5

Clinical and Vaccine Immunology
DOP : July 2007
• The specificities of the assay in this pediatric population were 79% and 85% using 60 pg/ml and 80 pg/ml as cutoff values.

• Use of BDG assay in pediatric population is limited by its further poor sensitivity and specificity as compared to adults.
A total of 838 patients (138 with proven or probable invasive fungal diseases), included in 6 studies, were analyzed.

The pooled sensitivity, specificity were 0.52, 0.58 respectively.

The accuracy of $(1 \rightarrow 3)$-β-D-glucan test in bronchoalveolar lavage fluid is poor.
• Total 37 CSF specimen

• Diagnostic performance was determined using 31 pg/ml cutoff

• Sensitivity was 96%, specificity 82%, positive and negative predictive value 93% and 90%

• scattered case reports of using CSF for diagnosing candida and aspergillosis meningitis
A multiple antibiotic exposed patient with culture negative polymorph predominant post neurosurgical meningitis

- CSF GM < 0.5
- CSF BDG >523
- Serum BDG=450

- Started on liposomal amphtericin 250 mg od for 4 weeks
- Then fluconazole 800mg and 6g flucytosine daily
- Improved well
False positive BDG

- Bacteremia
- Hemodialysis
- Beta-lactam antibiotics
- Surgical mesh
- IVIG
- IV albumin
Limitations of BDG

- High concentrations of **bilirubin** and **triglycerides** are inhibitory and would cause false-negative results, while **hemolysis** would cause false-positive results.

- Interference would be expected to occur at 72 mg/dl for bilirubin (which would be unlikely to occur clinically), and 466 mg/dl for triglycerides.

False positive BDG

- Hemodialysis with cellulose membranes
  - Exposure to cellulose containing dialysis membranes leads to false positive BDG assay but polysulphone membranes are used nowadays in major dialysis centers.

- Serosal exposure to gauze or other materials that contain glucans, such as
  - during surgery
  - administration of blood products
  - Albumin, immunoglobulin, coagulation factors, or plasma protein fraction filtered through BG-containing filters.
• Batches of the amoxicillin–clavulanic acid infusion fluid used during this period were positive for 1,3-β-D-glucan.
• 1,3-β-D-glucan (1339 pg/ml) was detected in the amoxicillin–clavulanic acid used to treat these patients.
• Given the difficulties encountered in the diagnosis of invasive fungal disease, it would be desirable to eliminate the fungal material from antibiotic agents.
Reactivity of (1→3)-\(\beta\)-d-Glucan Assay with Commonly Used Intravenous Antimicrobials

Francisco M. Marty,\(^{1,2,3}\*\) Colleen M. Lowry,\(^1\) Steven J. Lempitski,\(^4\) David W. Kubiak,\(^1\) Malcolm A. Finkelman,\(^4\) and Lindsey R. Baden\(^{1,2,3}\)

Brigham & Women’s Hospital,\(^1\) Dana-Farber Cancer Institute,\(^2\) Harvard Medical School,\(^3\) Boston, Massachusetts, and Associates of Cape Cod, Inc., East Falmouth, Massachusetts\(^4\)

Received 30 May 2006/Returned for modification 15 July 2006/Accepted 31 July 2006
Salient features

• Forty-four intravenous antimicrobials were tested for the presence of glucan (BG).

• Colistin, ertapenem, cefazolin, trimethoprim-sulfamethoxazole, cefotaxime, cefepime, and ampicillin-sulbactam tested positive for BG at reconstituted-vial concentrations

• But negative for BDG when diluted to usual maximum plasma concentrations.

• Cross-reactivity likely comes from contaminants during the manufacturing process rather than from the drug itself
Bacteremia causing BDG positive

**TABLE 1. Fungitell BG assay results for various patient groups**

<table>
<thead>
<tr>
<th>Group (no. of patients)</th>
<th>No. of patients with BG result(^a) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Blood donors (36)</td>
<td>0</td>
</tr>
<tr>
<td>Blood culture positive for yeast (15)</td>
<td>13</td>
</tr>
<tr>
<td>Blood culture positive for gram-positive cocci (15)</td>
<td>11(^b)</td>
</tr>
<tr>
<td>Blood culture positive for gram-negative bacilli (10)</td>
<td>3</td>
</tr>
<tr>
<td><em>Histoplasma</em> antigen positive (6)</td>
<td>6</td>
</tr>
<tr>
<td><em>Histoplasma</em> antigen negative (10)</td>
<td>2</td>
</tr>
<tr>
<td><em>Aspergillus</em> galactomannan positive (32)</td>
<td>31</td>
</tr>
<tr>
<td><em>Aspergillus</em> galactomannan negative (32)</td>
<td>9</td>
</tr>
</tbody>
</table>

\(^a\) Positive, \(\geq 80\) pg/ml; indeterminate, 60 to 79 pg/ml; negative, <60 pg/ml.

Total 43 patients with bacteremia were analysed.

For the 22 patients undergoing bacteremia due to Gram-negative bacilli, we observed 13 false-positive results.

Among the 17 patients with bloodstream infection involving Gram-positive cocci, three false-positive tests.

Beta-glucan levels were significantly higher in patients with Gram-negative bacilli bloodstream infection in comparison to those with bacteremia due to Gram-positive cocci.
Note

Can bacteraemia lead to false positive results in 1,3-beta-d-glucan test? Analysis of 83 bacteraemia episodes in high-risk patients for invasive fungal infections

Gökhan Metan\textsuperscript{a,*}, Ayse Nedret Koc\textsuperscript{b}, Çiğdem Ağkus\textsuperscript{a}, Leyla Gül Kaynar\textsuperscript{c}, Emine Alp\textsuperscript{a}, Bülent Eser\textsuperscript{c}

\textsuperscript{a} Department of Infectious Diseases, Faculty of Medicine, Erciyes University, Kayseri, Turkey
\textsuperscript{b} Department of Clinical Microbiology, Faculty of Medicine, Erciyes University, Kayseri, Turkey
\textsuperscript{c} Department of Hematology, Faculty of Medicine, Erciyes University, Kayseri, Turkey
• This study suggest that concurrent or recent bacteraemia very rarely leads to BDG reactivity.

• In these cases, other potential causes of false positivity should be ruled out (such as haemodialysis with cellulose membranes, treatment with immunoglobulin, albumin, or other blood products filtered through BDG-containing cellulose filters, serosal exposure to glucan containing gauze and administration of amoxicillin-clavulanic acid.)
Whether candida colonisation can raise BDG levels??

• It was found that Candida spp colonization alone did not appear to cause BG positivity however compared with patients without mucositis, those with mucositis (such as from chemoradiation) were seen to have higher BG levels.

Beta Glucan Assay vs Galactomannan assay

Use and Limits of (1-3)-β-D-Glucan Assay (Fungitell), Compared to Galactomannan Determination (Platelia Aspergillus), for Diagnosis of Invasive Aspergillosis

Annie Sulahian, a Raphael Porcher, b Anne Bergeron, c Sophie Touratier, d Emmanuel Raffoux, e Jean Menotti, a Francis Derouin, a Patricia Ribaud f

Journal of Clinical Microbiology
DOP: April 2014
• The GM assay was found to be more specific than the BG assay
• BG assay more sensitive than the GM assay for IA diagnosis.
• Combining GM and BG assays resulted in a very high diagnostic value for two positive results.
• Its use in combination with the GM test may strengthen IA diagnosis, but the additional costs due to the BG test are high and the overall benefit of such a combination remains limited.
Conclusions

• Beta Glucan Assay is a very good ‘rule out’ test as it has close to 100% negative predictive value.

• The test is not organism-specific and does not detect several types of fungal infections (Mucor and Crytococcus) so it should be used in conjunction with other forms of fungal testing.

• Avoid lipemic, hemolyzed or icteric specimens.
• A BDG assay cut-off of 80 pg/mL leads to a high number of false positive results in ICU patients
• cut-off of at least 144 pg/mL will be more specific for IC, although this may need further validation with larger trials.
• Discontinuation of empiric antifungal therapy based on a value < 80 resulted in a cost savings of 14000 INR (215$) per day of therapy per patient
• High specificity if >523
• High negative predictive value when <80
• Most useful when <80 or >523
• Recommend whenever IC is suspected, along with blood cultures
• Ideal if same day TAT
In summary

• Low PCT suggests Candida rather than bacteria as a cause of septic shock in the appropriate clinical setting

• Serum Aspergillus galactomannan essential adjunct in neutropenic host with suspected Aspergillosis
  – BAL GM helpful in all settings with a cut off of 1.0

• Serum beta d glucan
  – valuable adjunct in ruling out or increasing likelihood of disseminated fungal infection
  – most useful for invasive candidiasis in the ICU, order routinely with blood cultures
  – Best non-invasive marker for PCP

• Conditions causing false positives, clinical setting and pre test probability need to be looked at closely with all biomarkers
Thank you!
CIDSCon 2018
8th Annual Conference of Clinical Infectious Diseases Society India

16th | 17th | 18th August 2018

Venue: Christian Medical College, Vellore
Theme: Consolidating knowledge to impact patient care