Detection of (1→3)-β-D-Glucan as a Marker of Invasive Fungal Disease

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Invasive Fungal Disease (IFD)

- Fungal infection of otherwise sterile sites (e.g., blood, deep tissue, organs, etc).
- Most common fungal pathogens:
  - Overall incidence of IFD 14 – 27 cases/100,000 patients/year
- High risk patients include:
  - Hematopoietic stem cell transplantation (HSCT)
  - Solid Organ Transplantation
  - Neutropenia
  - Significant immunosuppression (e.g., malignancy, chemotherapy, etc.)
- High morbidity and mortality associated with delayed diagnosis and delayed initiation of antifungal therapy
Challenges associated with diagnosis of IFDs

- Non-specific clinical symptoms

  ![Imaging](image1)
  ![Histopathology](image2)
  ![Direct Microscopy and Culture](image3)

- Patient status may be prohibitive to invasive procedures

- Molecular methods

- Specific and Pan-Fungal Biomarkers: \((1\rightarrow3)-\beta-D-Glucan\) (BDG)

(1→3)-β-D-Glucan (BDG)

- Cell wall polysaccharide present in most fungi:
  - Aspergillus spp., Candida spp., Fusarium spp., P. jirovecii, etc.
  - Except: Mucorales (e.g., Mucor spp., Absidia spp.), Cryptococcus spp., Blastomyces spp.

- Multiple commercial BDG assays available
  - Fungitell (Asso. Cape Cod Inc., USA)
    - FDA approved as an aid for the diagnosis of deep-seated invasive fungal infections
    - Should be used in conjunction with other diagnostic procedures
The Fungitell BDG Assay: How Does It Work?

- Amebocyte granules contain components of the *Limulus* crab clotting cascade
- Two modes of activation:
  - Endotoxin
  - BDG
- Kinetic ELISA
  - Patient samples run in duplicate
  - Optical Density (OD) values read every 30 sec for 40 min

Collect horseshoe crab blood and isolate amebocytes

Endotoxin (LPS) → Activated Factor C

Factor B → Activated Factor B

Proclotting Enzyme → Clotting Enzyme

Boc-Leu-Gly-Arg-pNA (artificial substrate) → Boc-Leu-Gly-Arg + pNA

(1,3)-β-D-Glucan → Activated Factor G → Factor G

Chromophore excited by 405 nm λ

Standards in Duplicate

Each patient is run in duplicate

Pt #1: Positive

Pt #2: Uninterpretable; Optical Artifact

Pt #3: Negative

Quantitative Range: 31 – 500 pg/mL

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<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Indeterminate</th>
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<tr>
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<td>80 to &gt;500 pg/mL</td>
<td>&lt;60 pg/mL</td>
<td>60 – 79 pg/mL</td>
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BDG as a Biomarker for IFD

- Performance characteristic vary, largely dependent on patient risk factors
  - Testing should be restricted to patients at high risk for IFD
    - HSCT, SOT and patients with hematologic malignancy
    - Patients on select immunosuppressive drugs and biologic agents

<table>
<thead>
<tr>
<th>Patient Population</th>
<th>Mean % Sensitivity (95% CI)</th>
<th>Mean % Specificity (95% CI)</th>
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<tbody>
<tr>
<td>White et. al. 2019 Cancer</td>
<td>80 (74-89)</td>
<td>63 (64-88)</td>
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<tr>
<td>He et. al. 2015 ICH or other high-risk</td>
<td>78 (75-81)</td>
<td>81 (80-83)</td>
</tr>
<tr>
<td>Lu et. al. 2011 ICH or other high-risk</td>
<td>75 (67-82)</td>
<td>79 (61-90)</td>
</tr>
<tr>
<td>Karageorgopoulos et. al. 2011 ICH or other high-risk</td>
<td>77 (67-84)</td>
<td>85 (80-90)</td>
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- 2 consecutively positive BDG results may be used as a diagnostic marker for the presence of an IFD
- Absence of BDG antigenemia should be interpreted with caution!

BDG as a Biomarker for Pneumocystis Pneumonia

- Fungus that does not respond to antifungals
- Immunosuppressed patients are at greatest risk
- High mortality (10-60%) rate
- Diagnosis of *Pneumocystis* infection is challenging
- BDG is a major component of *P. jiroveci* cell wall
- 11 study meta-analysis evaluated performance of BDG for *P. jiroveci* in serum
  - Overall sensitivity: 95% (91-97%)
  - Overall specificity: 86% (82-90%)
  - Negative LR (0.06)
- Negative BDG result may be used to exclude *P. jiroveci* pneumonia
BDG Test Utilization Pearls

• BDG may be detected prior to symptom onset in critically ill patients
  • Single positive BDG result in at-risk patients warrants close monitoring and/or further evaluation

• Conflicting reports on the value of trending BDG levels to monitor response to antifungal therapy

• Documented causes of elevated BDG levels in serum:
  • IVIG, albumin
  • Gauze packing during surgery
  • Hemodialysis using cellulose containing membranes
  • Select antibiotics (e.g., Trimethoprim/sulfamethoxazole, amoxicillin/clavulanate) (?)
  • Bacteremia (?), severe mucositis, mucosal colonization with Candida spp.

References

Thank You