Assessment of Serum 1,3-β-D-glucan as a Measure of Disease Burden in Invasive Pulmonary Aspergillosis

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Study Objectives

• Two surrogate markers accepted by EORTC/MSG
  – Galactomannan & 1,3-β-D-glucan
  – Little clinical experience with 1,3-β-D-glucan in high risk patients for diagnosis of IPA

➢ Measure the serum kinetics of 1,3-β-D-glucan in murine and guinea pig models of IPA

➢ Examine assay as a surrogate marker of disease burden
  – Presence of antifungal therapy
Murine Model of Invasive Pulmonary Aspergillosis

5-week ICR mice

Pulmonary inoculation *A. fumigatus*

Antifungals initiated

Cortisone acetate
Cyclophosphamide

End of Survival arm

Survival Arm

D-2 D0 D+1 D+2 D+3 D+4 D+5 D+6 D+7 D+8

Blood collected and serum separated on days +1, +3, +5, +7 for β-glucan assay (serum kinetics)

Serum 1,3-β-D-Glucan Assay

- Endotoxin (LPS) → Activated Factor C
- Factor C (Eliminated from Fungitell kits) → Activated Factor C
- Factor B → Activated Factor B
- β-(1,3)-D-glucan → Activated Factor G

Proclotting Enzyme → Activated Clotting Enzyme


Chromogen (absorbs at 405 nm)

Associates of Cape Cod. Fungitell™ Product Insert.
Methods - Serum β-1,3-Glucan Assay

• Serum separated and transferred to 96 well cell culture tray
  – 5 mcL per well
  – Each sample in duplicate

• Alkaline serum treatment
  – Incubation 37°C

• Fungitell™ reagent
  – Mean rate ΔO.D. (405 nm) over 40 minute period

• Unknowns compared to standard curve
  – Range 0 - 500 pg/mL

Associates of Cape Cod. Fungitell™ Product Insert.
Methods - Pulmonary Fungal Burden

- Fungal burden
  - Lungs harvested at Day +5, homogenized and bead beaten
  - DNA extracted using proteinase K incubation and Qiagen DNeasy columns

- DNA analyzed by real-time qPCR assay using probes and primers specific for DNA encoding *Aspergillus FKS* gene

- Unknowns compared to standard curve generated from *Aspergillus* genomic DNA
  - Conidial equivalents/g

- Samples were run in duplicate

![Standard Curve Plot](r^2 = 0.9955)
Serum 1,3-β-D-Glucan Kinetics

![Graph showing serum β-glucan levels over time](image)

<table>
<thead>
<tr>
<th>Serum β-Glucan</th>
<th>Uninfected</th>
<th>Day +1</th>
<th>Day +3</th>
<th>Day +5</th>
<th>Day +7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>37.6</td>
<td>29.2</td>
<td>218</td>
<td>635</td>
<td>442</td>
</tr>
<tr>
<td>Range</td>
<td>56.0 - 68.3</td>
<td>12.3 - 39.2</td>
<td>49.2 - 609</td>
<td>142 - 1375</td>
<td>225 - 510</td>
</tr>
</tbody>
</table>

*P < 0.01 vs. Day +1, 1 hr SAC and Uninfected Controls
**Results - AF293**

<table>
<thead>
<tr>
<th>AF293</th>
<th>Control</th>
<th>AMBd 3 mg/kg IP</th>
<th>POS 40 mg/kg BID</th>
<th>LAMB 10 mg/kg IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Survival</td>
<td>20</td>
<td>50</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>(Median Survival)</td>
<td>(9 days)</td>
<td>(10 days)</td>
<td>(&gt;12 days)</td>
<td>(10.5 days)</td>
</tr>
<tr>
<td>Median β-glucan</td>
<td>758</td>
<td>250 ‡</td>
<td>28.5*</td>
<td>127∫</td>
</tr>
<tr>
<td>(Range)</td>
<td>(377 - 1426)</td>
<td>(63.8 - 525)</td>
<td>(0 - 111)</td>
<td>(28.5 - 259)</td>
</tr>
<tr>
<td>Median Log CE/gm</td>
<td>10</td>
<td>9.6</td>
<td>3.6*</td>
<td>8.9∫</td>
</tr>
<tr>
<td>(Range)</td>
<td>(9.1 - 11)</td>
<td>(8.8 - 11)</td>
<td>(0 - 8.7)</td>
<td>(8.3 - 9.8)</td>
</tr>
</tbody>
</table>

*P < 0.01 vs. Control, AMBd, & LAMB; ‡P < 0.01 vs. Control; ∫P < 0.01 vs. Control
## Results - CEA10

<table>
<thead>
<tr>
<th>CEA10</th>
<th>Control</th>
<th>AMBd 3 mg/kg IP</th>
<th>POS 40 mg/kg BID</th>
<th>LAMB 10 mg/kg IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percent Survival</strong></td>
<td>10</td>
<td>50</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>(Median Survival)</td>
<td>(7.5 days)</td>
<td>(11.5 days)</td>
<td>(&gt;12 days)</td>
<td>(10.5 days)</td>
</tr>
<tr>
<td><strong>Median β-glucan</strong></td>
<td>707</td>
<td>315‡</td>
<td>0*</td>
<td>167∫</td>
</tr>
<tr>
<td>(Range)</td>
<td>(328 - 959)</td>
<td>(115 - 1217)</td>
<td>(0 - 173)</td>
<td>(463 - 381)</td>
</tr>
<tr>
<td><strong>Median Log CE/gm</strong></td>
<td>9.6</td>
<td>9.0</td>
<td>7.3*</td>
<td>9.5</td>
</tr>
<tr>
<td>(Range)</td>
<td>(0 - 11)</td>
<td>(7.6 - 10)</td>
<td>(0 - 8.7)</td>
<td>(7.7 - 10)</td>
</tr>
</tbody>
</table>

* P < 0.01 vs. Control, AMBd, & LAMB; ‡ P = 0.01 vs. Control; ∫ P < 0.01 vs. Control
Guinea Pig Model of Invasive Pulmonary Aspergillosis

Antifungals initiated:
- Cortisone acetate
- Cyclophosphamide

Tissue Burden:
Animals euthanized; lungs harvested

Blood collected and serum separated on days +1, +3, +5, +7 for β-glucan assay (serum kinetics)
1,3-β-D-Glucan Kinetics in Guinea Pigs

**Serum β-Glucan**

<table>
<thead>
<tr>
<th></th>
<th>Uninfected</th>
<th>Day +1</th>
<th>Day +3</th>
<th>Day +5</th>
<th>Day +7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median</strong></td>
<td>0</td>
<td>5.0</td>
<td>0.9</td>
<td>47.6</td>
<td>1337</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0 - 43.9</td>
<td>0 - 40.6</td>
<td>0 - 60.5</td>
<td>0 - 971</td>
<td>294 - 1682</td>
</tr>
</tbody>
</table>

*P < 0.01 vs. Day +1, and Uninfected Controls*
# Results - Guinea Pigs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>POS 20 mg/kg BID</th>
<th>VOR 20 mg/kg BID</th>
<th>AMB 1.3 mg/kg IP</th>
<th>LAMB 10 mg/kg IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percent Survival</strong></td>
<td>0 (8)</td>
<td>75 (&gt;11)</td>
<td>37.5 (8)</td>
<td>0 (8)</td>
<td>0 (8.5)</td>
</tr>
<tr>
<td><strong>Median β-glucan</strong></td>
<td>326 (294 - 1337)</td>
<td>264 (158 - 371)</td>
<td>51.9 (0 - 941)</td>
<td>533 (520 - 1269)</td>
<td>25.0 (0 - 456)</td>
</tr>
<tr>
<td><strong>Median Log CE/gm</strong></td>
<td>7.7 (6.6 - 9.5)</td>
<td>4.2 (3.8 - 5.2)</td>
<td>4.8 (3.7 - 6.6)</td>
<td>7.0 (5.2 - 7.5)</td>
<td>7.0 (4.5 - 8.1)</td>
</tr>
</tbody>
</table>
Results - Serial Sampling

- **Posaconazole**
  - 
  - 
  - 
  - 60 pg/mL Threshold
  - Day 3, Day 5, Day 7, Day 11

- **Voriconazole**
  - 
  - 
  - 
  - 60 pg/mL Threshold
  - Day 3, Day 5, Day 7, Day 11

- **Amphotericin B**
  - 
  - 
  - 
  - 60 pg/mL Threshold
  - Day 3, Day 5, Day 7

- **Liposomal AMB**
  - 
  - 
  - 
  - 60 pg/mL Threshold
  - Day 3, Day 5, Day 7
Limitations

- False positives / interfering substances
  - Exposure to glucan-containing products (gauze)
  - Lipemia, hemolysis

- 1,3-β-D-glucan assay not evaluated with echinocandin therapy

- Discordance between serum 1,3-β-D-glucan and residual fungal burden

- No comparison with other surrogate markers
Conclusions

• Serum 1,3-β-D-glucan detectable earlier in course of infection in murine model
  – > 60 pg/mL by day +3
  – Later detection in guinea pig model (day 7)

• Early decreases in serum 1,3-β-D-glucan were predictive of survival in murine model
  – Median values of < 60 pg/mL on day +5 associated with 100% survival in posaconazole group
  – Reductions associated with improved survival in amphotericin B groups

• Median serum 1,3-β-D-glucan concentrations less predictive of outcome in guinea pigs
  – Reductions to < 60 pg/mL in serial samples in same animal from day 7 to day 11 observed in those treated with posaconazole
  – Increases in serial samples observed in animals who failed therapy
Conclusions

- Results suggest potential use of 1,3-β-D-glucan assay for screening and early diagnosis of IPA
  - Concern for discordance between residual tissue burden and serum 1,3-β-D-glucan concentrations

- Potential use of serial assessment of 1,3-β-D-glucan as a measure of treatment efficacy

- Additional pre-clinical studies warranted
  - Comparison with other surrogate markers
  - Clarify time required for assay to become positive