Utility of Surrogate Markers in Serum and BAL Fluid

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IAAM
INVASIVE ASPERGILLOSIS ANIMAL MODELS

AsTeC
ASPERGILLUS TECHNOLOGY CONSORTIUM
Background

- Early diagnosis of invasive pulmonary aspergillosis and initiation of antifungal therapy improves outcomes
  - Galactomannan and (1→3)-β-D-glucan within serum
- Utility of the galactomannan assay using bronchial alveolar lavage (BAL) fluid
- Reduced sensitivity of assays in the presence of antifungal therapy
Objectives

- Measure (1→3)-β-D-glucan and galactomannan within the BAL fluid in a guinea pig model of invasive pulmonary aspergillosis
  - Compare to results in serum

- Assess the utility of these assays in the presence of antifungal therapy
Guinea Pig Model of Invasive Pulmonary Aspergillosis

Guinea pigs ~500g

Pulmonary inoculation AF 293

Antifungals initiated

Antifungals Discontinued

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

Guinea Pig Model of Invasive Pulmonary Aspergillosis

Cortisone acetate Cyclophosphamide

Cortisone acetate Cyclophosphamide

D+9 D+10 D+11

Tissue Burden:
Animals euthanized; lungs harvested

Serum & BAL collected at 1 hr & days +3, +5, +7 for β-glucan & GM assays (untreated animals)

Serum collected on day +7 in those receiving antifungal therapy:
Control
PSC 20 mg/kg po BID
VRC 20 mg/kg po BID
AMBd 1.3 mg/kg IP QD
LAMB 10 mg/kg IP QD

(1→3)-β-D-glucan and Galactomannan Assays

**Fungitell (1→3)-β-D-glucan Assay**
- Transferred to 96 well cell culture plate in duplicate
- Mean rate ΔO.D. (405 nm) over 40 minute period
- Unknowns compared to standard curve

**Platellia Aspergillus EIA**
- Samples treated with EDTA acid solution, heat treated, and transferred to microwells containing conjugate and EBA-2 antibody
- Optical density of sample, positive control, negative control, and cut-off control measured (450 and 630 nm)
- GMI calculated as OD of each sample divided by mean cut-off of control OD
(1→3)-β-D-glucan
(Serum vs. BAL)
Galactomannan
(Serum vs. BAL)
### Results – Survival & Fungal Burden

<table>
<thead>
<tr>
<th>TX Group</th>
<th>Control</th>
<th>PSC</th>
<th>VRC</th>
<th>AMBd</th>
<th>LAMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Survival</td>
<td>9 days</td>
<td>&gt;11 days</td>
<td>8 days</td>
<td>9 days</td>
<td>9 days</td>
</tr>
<tr>
<td>p = 0.02</td>
<td>p = 0.03</td>
<td>37%</td>
<td>6%</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>Percent Survival</td>
<td>37%</td>
<td>81%</td>
<td>37%</td>
<td>6%</td>
<td>25%</td>
</tr>
<tr>
<td>Beta-glucan, pg/mL (range)</td>
<td>1407 (326 – 1682)</td>
<td>12.4 (0 – 371)</td>
<td>51.9 (0 – 941)</td>
<td>772 (520 – 1269)</td>
<td>25 (0 – 456)</td>
</tr>
<tr>
<td>GMI (range)</td>
<td>21.1 (0.64 – 39.6)</td>
<td>0.48 (0.11 – 0.62)</td>
<td>0.44 (0.23 – 0.60)</td>
<td>13.7 (3.76 – 35.1)</td>
<td>3.16 (0.5 – 23.5)</td>
</tr>
</tbody>
</table>
Conclusions

- Biomarkers detectable early in course of infection in BAL fluid versus serum
  - $(1\rightarrow 3)\beta$-D-glucan > 60 pg/mL by day +3
  - GMI > 0.5 by day +3
  - Day 5 – 7 for biomarkers in serum

- Mixed association between $(1\rightarrow 3)\beta$-D-glucan / galactomannan and survival with antifungal exposure
  - Changes in $(1\rightarrow 3)\beta$-D-glucan were predictive of survival in animals treated with posaconazole and AMBd, but not voriconazole and LAMB
  - Sensitivity of the galactomannan assay reduced with voriconazole exposure