Reconsidering the role of antibody testing in the diagnosis of invasive aspergillosis

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AsTec Meeting
November 16, 2009
Measuring antibody responses against *Aspergillus fumigatus* proteins among patients with invasive aspergillosis
Introduction

- Identifying subgroups of patients at particularly high-risk for developing invasive aspergillosis (IA) is a major priority.
- Even in high-risk populations, the relatively low prevalence of IA limits the positive predictive value (PPV) of screening tests.
  - Maximizing negative predictive value (NPV)
Introduction

- Serum IgG responses against *A. fumigatus* catalase at the time of hospital admission for HSCT or treatment of hematologic malignancy were 78% sensitive and 74% specific in identifying patients who subsequently developed IA (Sarfati, 2006)
  - NPV: 95% in population with 15% prevalence of IA
- 76% of patients would test negative at baseline
Hypothesis

• Negative serum IgG responses against certain *A. fumigatus* proteins measured prior to HSCT or chemotherapy for a hematologic malignancy will identify patients who are unlikely to subsequently develop IA.
Objectives

• To measure serum IgG responses against immunogenic *A. fumigatus* proteins among HSCT recipients and patients with hematologic malignancies
  – Baseline prior to HSCT or chemotherapy
  – At time of diagnosis and 4 weeks following the diagnosis of IA
Measuring baseline serum IgG responses

- Sera collected prior to HSCT or chemotherapy from 19 patients who subsequently developed proven or probable IA due to *A. fumigatus*
  - 16/19 HSCT
  - No evidence of prior colonization or infection with *A. fumigatus*
- 54 control patients undergoing HSCT or receiving chemotherapy at the same time who did not develop IA or colonization
- ELISA against 6 purified recombinant *A. fumigatus* proteins identified in a screening study
  - Extrapolation of concentrations from standard curve
Measuring baseline serum IgG responses

• All patients received fluconazole prophylaxis

• Median time to IA: 26 days (2-322)
  – 68% (13/19) within 30 days (early-onset)
  – 32% (6/19) after 60 days (late-onset)

• 47% (9/19) died, 53% (10/19) alive at follow-up ≥ 1 year
Performance of baseline IgG responses in identifying patients who develop IA

<table>
<thead>
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<th>Protein</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>p-value</th>
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<td>56</td>
<td>0.003</td>
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<th>p-value</th>
<th>PPV</th>
<th>NPV</th>
<th>Anticipated negative baseline test</th>
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Measuring serial serum IgG responses among patients with IA

- For 19 patients with IA, paired baseline serum and serum from time of diagnosis (acute serum) were collected
- For 13 patients, baseline, acute and serum from 4 weeks after the diagnosis of IA were collected
- No significant differences in median or mean IgG concentrations against any of the proteins across the time points
Measuring serial serum IgG responses among patients with IA.

**IgG responses against AF1 among IA patients who lived**

**IgG responses against AF1 among IA patients who died**

**IgG responses against AF2 among IA patients who lived**

**IgG responses against AF2 among IA patients who died**
Measuring serial serum IgG responses among patients with IA

IgG responses against AF3 among IA patients who lived

Percent change in IgG compared to baseline

Baseline 4 weeks

IgG responses against AF3 among IA patients who lived
## IgG responses at week 4 and outcome of IA

<table>
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<th>Protein</th>
<th>Patients who lived</th>
<th>Patients who died</th>
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<td>60% (3/5)</td>
<td>40% (2/5)</td>
<td>56% (5/9)</td>
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<tr>
<td>AF13</td>
<td>80% (4/5)</td>
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<td>AF1</td>
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Conclusions

• Baseline serum IgG responses against *A. fumigatus* proteins prior to HSCT or chemotherapy were higher among patients who subsequently developed IA than controls
  – Some patients may be infected or colonized with *A. fumigatus* at the time of HSCT/chemotherapy
    • IA may result from progression of infection/colonization rather than acute inhalation of conidia
Conclusions

• Negative baseline serum IgG concentrations against *A. fumigatus* proteins may be useful for identifying a subgroup of HSCT recipients and hematologic malignancy patients at very low risk for IA
  – NPV: 95% in population with 15% prevalence of IA
Conclusions

- Measuring baseline IgG responses against a combination of proteins may result in more negative tests at baseline without significantly changing NPV
  - 65% of patients anticipated to test negative
Conclusions

• Increased IgG responses against *A. fumigatus* proteins at 4 weeks after the diagnosis of IA compared to baseline may identify patients with increased likelihood of survival
  – Increased IgG responses may be markers for other determinants of good outcome
  – Immune responses against one or more proteins may contribute to the resolution of IA
• Therapeutic or vaccine targets
Conclusions

• Further studies of the role of antibody testing in identifying patients at risk for IA or diagnosing pts with IA are warranted.
Future directions

• Verify preliminary findings in larger studies
  – HSCT at University of Florida and UPMC

• Study other high-risk populations
  – Lung transplant at UPMC

• Proteomic screening
  – Collaboration with Phil Felgner on R21/R33 application
Acknowledgements

• NIH/NIAID PO1: Mycology Research Unit Program Project (Nguyen, PI)
  – John Wingard, Clinical Core and Repository
  – Hong Nguyen, University of Pittsburgh
  – Neil Clancy, University of Pittsburgh
  – Haoping Liu and Phil Felgner, University of California-Irvine
  – Jim Cutler, Louisiana State University

• Rory Duncan, Dennis Dixon, NIAID
Conclusions

• Decisions about prophylactic strategies based upon antibody screening would have to weigh potential benefit of avoiding unnecessary antifungal therapy with the consequences of false-positive and –negative tests
  – Baseline anti-AF11 testing of 1000 HSCT recipients
    • Administer prophylaxis to 126 patients who would develop IA
    • Avoid antifungal therapy in 476 who would not develop IA
    • Fail to give prophylaxis to 24 patients with IA
    • Administer unnecessary prophylaxis to 374 patients