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

[1]Neil Clancy, M.D.
[2]M. Hong Nguyen, M.D.
Directors, Mycology Research Unit[1] and
Transplant Infectious Diseases Program[2],
University of Pittsburgh Medical Center

John Wingard, M.D.
Director, Bone Marrow Transplant Program,
University of Florida College of Medicine

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

Protein	Sensitivity	Specificity	p-value
AF11	84	56	0.003
AF13	74	59	0.02
AF1	67	65	0.03
AF2	72	56	0.06
AF3	67	65	0.02
AF4	78	52	0.05
AF11 or AF3	72	72	0.001
CAT	78	74	N/S

Performance of baseline IgG responses in identifying patients who developed IA

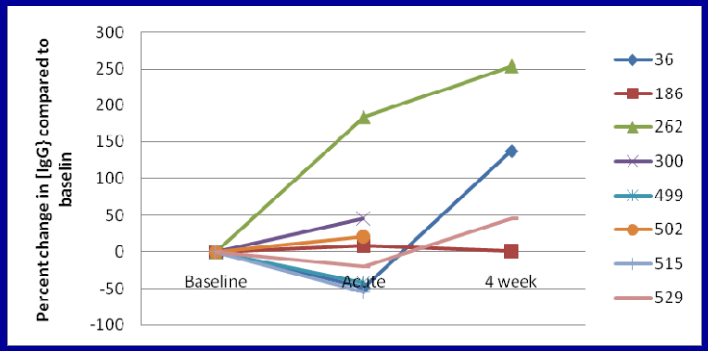
Protein	Sensitivity	Specificity	p-value	PPV	NPV	Anticipated negative baseline test
AF11	**84**	**56**	**0.003**	**25**	**95**	**50%**
AF13	74	59	0.02			
AF1	67	65	0.03			
AF2	72	56	0.06			
AF3	67	65	0.02			
AF4	78	52	0.05			
AF11 or AF3	**72**	**72**	**0.001**	**31**	**94**	**65%**
CAT	78	74	N/S	35	95	76%

Measuring serial serum IgG responses among patients with IA

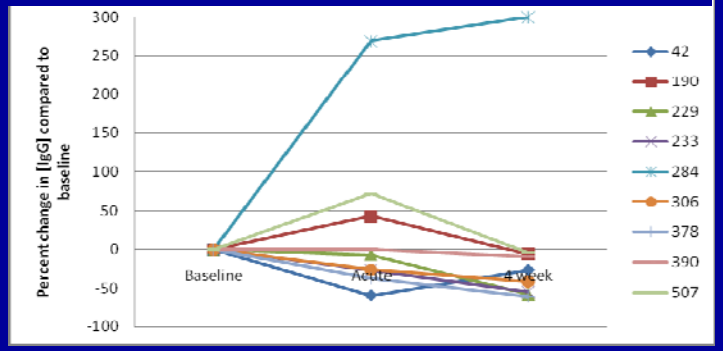
- For 19 patients with IA, paired baseline serum and serum from time of diagnosis (acute serum) were collected
- For 13 patients, baseline, acute and serum from 4 weeks after the diagnosis of IA were collected
- No significant differences in median or mean IgG concentrations against any of the proteins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

Measuring serial serum IgG responses among patients with IA

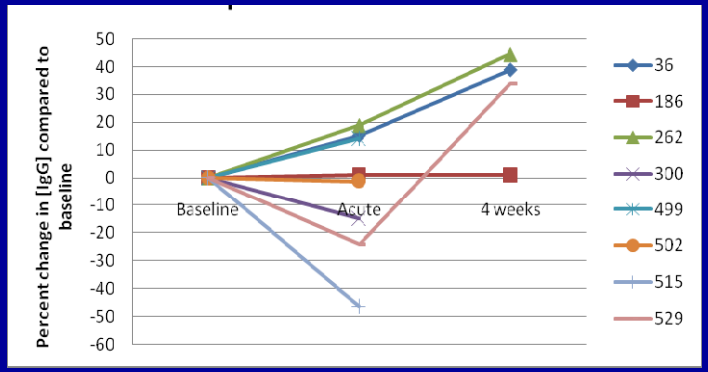
IgG responses against AF1 among IA patients who lived



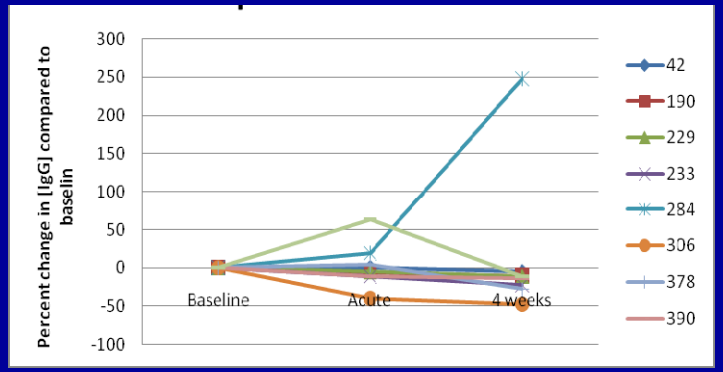
IgG responses against AF1 among IA patients who died



IgG responses against AF2 among IA patients who lived

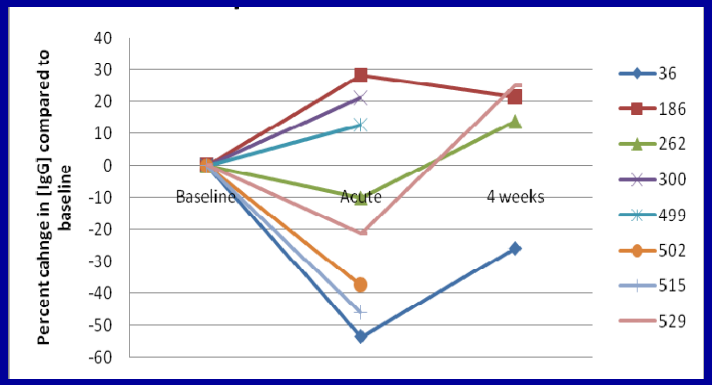


IgG responses against AF2 among IA patients who died

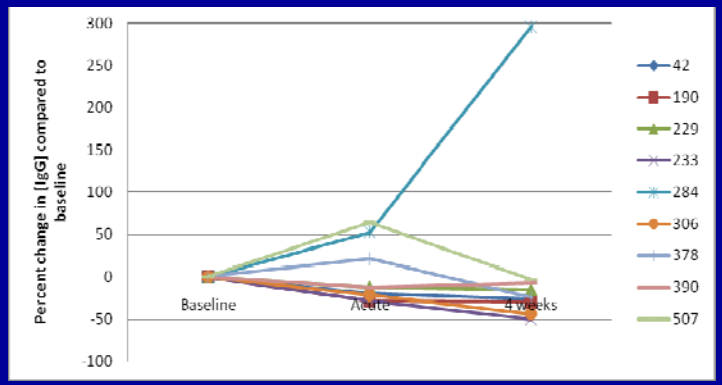


Measuring serial serum IgG responses among patients with IA

IgG responses against AF3 among IA patients who lived



IgG responses against AF3 among IA patients who lived



IgG responses at week 4 and outcome of IA

Protein	**Patients who lived**		**Patients who died**		p-value
	IgG increased	IgG not increased	IgG increased	IgG not increased	
AF11	60% (3/5)	40% (2/5)	56% (5/9)	44% (4/9)	NS
AF13	80% (4/5)	20% (1/5)	33% (3/9)	67% (6/9)	0.26
AF1	**75% (3/4)**	**25% (1/4)**	**11% (1/9)**	**89% (8/9)**	**0.05**
AF2	**75% (3/4)**	**25% (1/4)**	**11% (1/9)**	**89% (8/9)**	**0.05**
AF3	**75% (3/4)**	**25% (1/4)**	**11% (1/9)**	**89% (8/9)**	**0.05**
AF4	75% (3/4)	25% (1/4)	33% (3/9)	67% (6/9)	0.27

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 prophlyaxis to 24 patients with IA
    - Administer unneccessary prophylaxis to 374 patients