Serum IgG Responses against *Aspergillus* Proteins before Hematopoietic Stem Cell Transplantation or Chemotherapy Identify Patients Who Develop Invasive Aspergillosis

Chen Du,1 John R. Wingard,2 Shaoji Cheng,1 M. Hong Nguyen,1 Cornelius J. Clancy1,3

The ability to identify patients at particularly low risk for invasive aspergillosis (IA) would facilitate more efficient targeting of antifungal prophylaxis. We measured baseline serum immunoglobulin responses against 6 purified recombinant *Aspergillus fumigatus* proteins before hematopoietic stem cell transplantation (HSCT) or chemotherapy in 73 subjects, including 19 patients who subsequently developed proven or probable IA and 54 uninfected controls. We also assessed responses at the time of IA diagnosis and 4 weeks later (acute and convalescent sera, respectively). Baseline IgG responses against enolase, Ahp1, Hsp90, Crf1, and Cdc37 were significantly higher in the patients with IA compared with controls (P < .05). Cutoff concentrations identified by receiver-operating characteristic curve analysis were 67%-84% sensitive and 52%-67% specific. In a population with a 15% likelihood of developing IA, positive and negative predictive values would be 22%-26% and 92%-95%, respectively. Positive IgG responses against Hsp90, Pep2, Crf1, and Cdc37 were specifically associated with early-onset IA (<40 days) rather than late-onset IA (P ≤ .009). Increased IgG concentrations against Hsp90, Pep2, and Crf1 in convalescent sera versus baseline sera were more likely in the patients with IA who survived (P ≤ .01). IgG responses in acute sera were not correlated with outcomes, and IgM and IgA responses did not differ in baseline, acute, or convalescent sera between the patients and controls. In conclusion, baseline IgG responses against *Aspergillus* proteins may be useful screening tests for patients at low risk for IA. Our data suggest that some patients with IA have significant colonization or ongoing *Aspergillus* infections before immunosuppression. As such, IA may reflect unique predispositions to infection and/or progression from endogenous sources.

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**KEY WORDS:** Antibody, Diagnosis, Invasive fungal infections

**INTRODUCTION**

Approximately 15%-20% of hematopoietic stem cell transplantation (HSCT) recipients and patients receiving chemotherapy for hematologic malignancies develop invasive aspergillosis (IA) [1-4]. IA can develop either early (<40 days) or late (>40 days) after HSCT [2,3]. Underlying diseases and transplantation-related immunosuppression are risk factors for early-onset IA, whereas such factors as steroid use and chronic graft-versus-host disease are major risks for late-onset IA [2,3]. Case fatality rates for both early and late IA are as high as 50%-90% despite antifungal therapy [1-5]. The prompt treatment of IA improves survival [6-8], but timely and accurate diagnosis is often difficult.

Given the severity of IA and the challenges of establishing a definite diagnosis, prophylactic antifungal therapy is often administered to patients considered at high risk for IA [9,10]. Even in the highest-risk populations, however, preventive strategies are limited by the low prevalence of the disease, which dictates that large numbers of patients must be exposed to an intervention to prevent a small number of cases [6,7]. In these scenarios, economic costs and the risks of drug toxicity, untoward drug–drug interactions, and...
emergence of drug resistance may outweigh the benefits of preventing IA. Not surprisingly, there is great interest in devising methods of identifying subgroups at particularly high risk for developing IA. Screening tests that stratify patients before transplantation or immunosuppression, such as genetic testing for deficiencies in components of innate immunity, are currently in development, but none has yet been introduced to clinical practice [11-14]. When developing screening strategies for IA, it is important to recognize that the positive predictive value (PPV) of any test, even one with good sensitivity and specificity, will be limited by the low prevalence of the disease. As such, it has been suggested that the objective of screening should be to maximize the negative predictive value (NPV) [6,7], thereby reliably identifying low-risk patients who are unlikely to develop IA.

Along these lines, an earlier study demonstrated that measurements of serum IgG responses against *Aspergillus fumigatus* catalase at the time of hospital admission for HSCT or treatment of a hematologic malignancy were 78% sensitive and 74% specific in identifying patients who later developed IA [15]. Based on these figures, the anticipated NPV of baseline antibody testing would be 95% in a population with a 15% prevalence of IA and even higher in populations with a lower prevalence. These data suggest that baseline antibody testing against *A. fumigatus* proteins may be a useful screening strategy for IA in relatively high-risk populations. The primary objective of the present study was to compare baseline IgG responses against purified recombinant *A. fumigatus* proteins in the sera of HSCT recipients and patients with hematologic malignancy who subsequently developed IA and those who did not (negative controls). We also sought to correlate serum IgG responses with the outcomes of IA.

**MATERIALS AND METHODS**

**Patient Population and Definitions**

Patients with acute leukemia undergoing HSCT or receiving chemotherapy who consented to inclusion in our Mycology Research Unit repository were eligible for this study, which was approved by and performed according to guidelines of the Institutional Review Board at the University of Florida. Baseline blood samples were obtained before HSCT or receipt of chemotherapy. These patients were followed prospectively for a minimum of 1 year or until death. In the patients who developed IA, blood samples were also collected on the day of diagnosis (acute serum) and 4-6 weeks later (convalescent serum). Surviving patients were followed for at least 6 weeks after the diagnosis of IA [16]. IA was classified as proven or probable using the European Organization for Research and Treatment of Cancer–Mycoses Study Group criteria [17]. Early-onset and late-onset IA were defined as cases developing within 40 days of HSCT and after 40 days of HSCT, respectively [3]. Patients receiving chemotherapy who developed IA were considered to have early-onset IA. Outcomes of IA were defined according to European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC-MSG) criteria [18].

**Determination of Immunoglobulin Responses to Aspergillus Proteins**

We performed ELISA against 6 recombinant proteins. Five proteins (enolase, Ahp1, Hsp90, Pep2, and Crf1) were chosen because they were identified by a study that screened a cDNA library with sera from rabbits experimentally infected with *A. fumigatus* [19]. Each of these proteins has been shown to react with antibodies in human sera. Enolase, Ahp1, and Crf1 are known allergens that elicit IgE responses in patients with allergic aspergillosis [20-23]. Anti-Hsp90 and anti-Pep2 antibodies are found in the sera of patients with various types of aspergillosis [15,24,25]. Of note, *Candida albicans* enolase and Hsp90 induce protective immune responses against systemic candidiasis in both mice and humans, respectively [26-31]. Moreover, human recombinant monoclonal antibody against an Hsp90 epitope was shown in a clinical trial to improve the efficacy of lipid formulation amphotericin B against invasive candidiasis [28]. Finally, we selected Cdc37 as the sixth protein, because it is an Hsp90 co-chaperone (http://www.ncbi.nlm.nih.gov/gene/8048582), which was reactive with antibodies in human sera with IA in a pilot screen.

**Expression and Purification of Aspergillus Recombinant Proteins**

*A. fumigatus* (AF 237) was maintained on BBL Malt Agar medium (BD Diagnostic Systems, Sparks, MD). For RNA isolation, *A. fumigatus* was first cultured on a BBL Malt Agar medium slant for 5 days. A conidial suspension was then prepared by washing the surface with PBS including 0.05% Tween 20 and then filtering through Calbiochem Miracloth (EMD Millipore, Billerica, MA) to remove debris and hyphae. A suspension of 1 × 10^8 spores/mL of *A. fumigatus* was inoculated in 100 mL of YG medium (0.5% yeast extract and 2% glucose), which was incubated subsequently at 37°C. After 16 hours, hyphae were harvested by filtration through #1 Whatman filter paper, quick-frozen in liquid nitrogen, and disrupted by grinding. Total RNA from mycelia was extracted using an Ambion RNA Extraction Kit for Yeast (Life Technologies, Carlsbad, CA).
Synthesis of first-strand cDNA was performed with 1 μg of total RNA using the Improm-II Reverse-Transcription System (Promega, Madison, WI). Then 2 μL of cDNA from the reverse-transcription reactions were used for subsequent PCR (Phusion Flash High-Fidelity PCR Master Mix; New England BioLabs, Ipswich, MA) with gene-specific primers to obtain the full-length or partial cDNA (Table 1). The final primer concentration was 0.5 μM. Cycling conditions for 2-step PCR were initial denaturation at 98°C for 10 seconds, denaturation at 98°C for 1 second, extension at 72°C for 15 seconds/ kb; and final extension at 72°C for 5 minutes.

Gel-purified PCR products (A. fumigatus cDNA encoding each protein) were cloned into pET-32 Ek/LIC vector (Novagen; EMD Millipore), which was prepared for high-level expression of polypeptides. Fusion proteins contained a cleavable His-Tag for detection and purification. The annealed LIC vector and insert were transformed into competent Escherichia coli BL21 (DE3) (Novagen), and inserts were confirmed by colony-PCR and sequencing. An Ahp1 (168-aa peptide) expression strain was kindly provided by Dr. Utz Reichard [19]. Isopropyl β-D-1-thiogalactopyranoside (1 mM) was used to induce the maximum expression of desired proteins in E. coli. Cells from overnight liquid culture were harvested by centrifugation, and the cell pellet was suspended at room temperature in BugBuster Protein Extraction Reagent containing Benzonase Nuclease (125 U/g cell paste; Novagen). After a 20-minute incubation at room temperature, the insoluble cell debris was removed by centrifugation. The pellet was saved for inclusion body extraction. The remaining soluble extract was subjected to subsequent purification with a His-Bind Purification Kit (Novagen). For Cdc37, Hsp90, and Pep2, pilot experiments showed that most of the expressed protein was included in the insoluble inclusion body. Inclusion body purification was performed by incubation with 1 x binding buffer containing 1 KU/mL lysozyme and column purification under denaturing conditions (with 6 M urea included in the binding buffer and elution buffer). The purity of the proteins was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blot analysis with an antipolyhistidine monoclonal antibody.

ELISA

All protein antigens were coated overnight at 4°C on ELISA plates (EIA/RIA plates; Corning, Corning, NY) at a coating buffer concentration of 1 μg/mL. After washing, the wells were filled with 300 μL of 10% nonfat dry milk in PBS supplemented with 0.25% Tween 20 (PBST). After a 2-hour incubation at room temperature, patient serum diluted 1:100 in PBST was added to each well. The plates were

<table>
<thead>
<tr>
<th>Table 1. Primers for Expression of A. fumigatus Proteins</th>
<th>Protein</th>
<th>Sense Primer (5’-3’)</th>
<th>Antisense Primer (3’-5’)</th>
</tr>
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<tbody>
<tr>
<td>Enolase</td>
<td>GAGGAGAGGAGCCTGTCTTACTCACCTTGAAGGAGAAGCCCGGTTACAGGTTGACGGCAGTGCG</td>
<td>GAGGAGAGGAGCCTGTCTTACTCACCTTGAAGGAGAAGCCCGGTTACAGGTTGACGGCAGTGCG</td>
<td></td>
</tr>
<tr>
<td>Ahp1</td>
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<td>GAGGAGAGGAGCCTGTCTTACTCACCTTGAAGGAGAAGCCCGGTTACAGGTTGACGGCAGTGCG</td>
<td></td>
</tr>
<tr>
<td>Hsp90</td>
<td>GAGGAGAGGAGCCTGTCTTACTCACCTTGAAGGAGAAGCCCGGTTACAGGTTGACGGCAGTGCG</td>
<td>GAGGAGAGGAGCCTGTCTTACTCACCTTGAAGGAGAAGCCCGGTTACAGGTTGACGGCAGTGCG</td>
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</tr>
<tr>
<td>Pep2</td>
<td>GAGGAGAGGAGCCTGTCTTACTCACCTTGAAGGAGAAGCCCGGTTACAGGTTGACGGCAGTGCG</td>
<td>GAGGAGAGGAGCCTGTCTTACTCACCTTGAAGGAGAAGCCCGGTTACAGGTTGACGGCAGTGCG</td>
<td></td>
</tr>
<tr>
<td>Cdc37</td>
<td>GAGGAGAGGAGCCTGTCTTACTCACCTTGAAGGAGAAGCCCGGTTACAGGTTGACGGCAGTGCG</td>
<td>GAGGAGAGGAGCCTGTCTTACTCACCTTGAAGGAGAAGCCCGGTTACAGGTTGACGGCAGTGCG</td>
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Antibody Response in Patients with Invasive Aspergillosis
incubated for 1 hour at 37°C and then washed 4 times with PBST. Secondary HRP-conjugated anti-IgG, -IgM, or -IgA antibody (MP Biomedicals, Solon, Ohio) was diluted 1:1000 and then added to each well. After a 1-hour incubation, the plates were washed three times with PBST and then with PBS, and then developed with o-phenylenediamine. The developing reaction was stopped with 1 M phosphoric acid. Optical densities were determined using a spectrophotometer at 450 nm. Background was defined in wells coated with the protein to which the secondary antibody was added but the primary antibody was not. All serum samples were tested in duplicate.

A standard curve was constructed using plates coated with 100 μL of 1 μg/mL mouse anti-human lambda antibody (Southern Biotech, Birmingham, AL) and purified human IgG solution (concentrations ranging from 10-1250 ng/mL; MP Biomedicals). A standard curve was plotted as standard IgG concentration versus the corresponding mean optical density value of triplicates. The concentrations of the putative immunoglobulin-containing samples were then interpolated from the standard curve.

**Statistical Analyses**

Median and interquartile IgG, IgM, and IgA concentrations against individual antigens were calculated, and differences were assessed using the Mann-Whitney test; statistical significance was set at \( P < .05 \). Sensitivity and specificity were calculated for antibodies against each antigen. Optimal cutoffs were determined by receiver-operating characteristic curve analyses. Positive and negative predictive values were calculated based on an IA rate of 15%.

**RESULTS**

**Baseline Serum IgG Responses against Recombinant *A. fumigatus* Proteins**

Seventy-three patients were enrolled, including 19 patients with IA and 54 uninfected controls. Sixteen of the 19 patients with IA (84%) had undergone HSCT, and the other 3 (16%) had received chemotherapy for hematologic malignancy. The corresponding numbers for the uninfected controls were 53 of 54 (98%) and 1 (2%). Among the patients with IA, 16 (84%) had invasive pulmonary aspergillosis and 3 (16%) had disseminated aspergillosis. The median time from HSCT to IA was 26 days (range, 2-322 days). Thirteen patients (68%) had early-onset IA. Eighteen of the 19 cases of IA were caused by *A. fumigatus*, and 1 case was caused by *A. niger*. All patients with IA and controls received fluconazole as antifungal prophylaxis.

Baseline serum IgG, IgM, and IgA concentrations were measured against purified recombinant enolase and Ahp1 in all patients and controls immediately before HSCT or institution of chemotherapy. Because of a shortage of serum from 1 patient with late-onset IA, immunoglobulin concentrations against Hsp90, Pep2, Crf1, and Cdc37 were determined for 18 patients and 54 controls.

There was significant intrasubject and intersubject variability in baseline IgG concentrations among both patients with IA and controls. In other words, the relative IgG concentrations in the serum of most subjects were not uniform against the panel of recombinant proteins. However, there was some correlation between responses against enolase, Ahp1, and Hsp90 \( (R^2 = 0.84) \); Hsp90 and Pep2 \( (R^2 = 0.86) \); and Crf1 and Cdc37 \( (R^2 = 0.84) \).

Baseline IgG concentrations against enolase, Ahp1, Hsp90, Crf1, and Cdc37 were significantly higher in the patients with IA compared with controls (Table 2). By receiver-operating characteristic curve analysis, we assigned cutoff IgG concentrations that best discriminated the patients with IA from controls (data not shown). The sensitivities and specificities of the cutoff concentrations in identifying patients who subsequently developed IA are presented in Table 2. Positive test results against enolase, Ahp1, Hsp90, Crf1, and Cdc37 were significantly associated with the development of IA. We also assessed combinations of 2 or more proteins, but results were not superior to those of the best proteins tested singly.

**Baseline Serum IgG Responses among Patients with Early-Onset and Late-Onset IA**

Baseline IgG concentrations against enolase, Hsp90, Pep2, Crf1, and Cdc37 were significantly higher in patients with early-onset IA than in controls. Baseline IgG concentrations were similar in patients with late-onset IA and controls. In a direct comparison of baseline sera in patients with early-onset IA and those with late-onset IA, positive IgG responses against Hsp90, Pep2, Crf1, and Cdc37 were specifically associated with early-onset disease (Table 2). Indeed, 100% (13 of 13) of patients who developed early-onset IA had a positive baseline IgG response against at least 2 proteins from among Hsp90, Pep2, Crf1, and Cdc37, compared with 20% (1 of 5) of patients with late-onset IA \( (P = .001) \). Positive baseline IgG responses against at least 3 of these proteins were positive in 85% (11 of 13) of patients with early-onset IA and in 20% (1 of 5) of those with late-onset IA \( (P = .002) \). Baseline IgG responses against enolase and Ahp1 were similar in patients with early-onset and late-onset disease.

**Associations between Serum IgG Response and Outcomes of IA**

Serum was available from the time of diagnosis of IA (acute serum) in 100% (19 of 19) of the patients...
and at 4 weeks after diagnosis (convalescent serum) in 68% (13 of 19) of patients. Similar to baseline, there was significant intrasubject and intersubject variability in IgG concentrations in acute and convalescent sera. Unlike at baseline, however, acute serum IgG responses did not differ between patients with IA and controls, and were not correlated with the time to onset of IA (data not shown). Overall, baseline and acute serum IgG concentrations against any of the recombinant proteins were not significantly different between the 47% (9 of 19) of patients who lived and the 53% (10 of 19) who died after the diagnosis of IA.

Among the patients with convalescent samples, 31% (4 of 13) lived and 69% (9 of 13) died. Patients who lived after being diagnosed with IA were more likely than patients who died, which demonstrated increased serum IgG concentrations against Hsp90, Pep2, and Crf1 in convalescent serum compared with baseline (Table 3).

**Serum IgM and IgA Responses against Recombinant Proteins**

There were no significant differences in IgM and IgA responses in baseline, acute, or convalescent sera between the patients with IA and controls.

**DISCUSSION**

This study is most notable for 2 findings. First, baseline serum IgG concentrations against recombinant *A fumigatus* enolase, Hsp90, Pep2, Crf1, and Cdc37 at the time of HSCT or chemotherapy for a hematologic malignancy were significantly higher in patients who subsequently developed proven or probable IA than in control patients who did not. In contrast, baseline IgM or IgA responses were not correlated with IA. Second, positive baseline IgG responses against at least 2 proteins among a panel consisting of Hsp90, Pep2, Crf1, and Cdc37 were specifically associated with early-onset rather than late-onset IA. By capturing baseline anti-*Aspergillus* antibody responses, our data provide a snapshot of the history of the host–pathogen interaction at the critical stage immediately before the onset of immunosuppression. This study is important for both the insight that it provides into the pathogenesis of IA and its potential implications for clinical practice.

Our findings indicate that in at least some patients, IA occurs in the context of an ongoing interaction between *Aspergillus* and the host immune system that antedates HSCT or the administration of chemotherapy. Along these lines, other studies have also reported elevated baseline serum IgG responses against purified recombinant *A fumigatus* proteins in patients with IA. In one study, approximately one-half of HSCT recipients and patients with hematologic malignancy with IA demonstrated high IgG titers against *A fumigatus* catalase and dipeptidyl-peptidase V DDPV on hospital admission [15]. In another study, 6 bone marrow transplant recipients with IA had positive baseline IgG responses against Pep2 and 3 other proteins (superoxide dismutase, a metalloprotease, and a 94-kDa protein), although these responses were not compared with those in controls [25]. The elevated baseline IgG responses against recombinant proteins in those...
studies and ours suggest that some patients with IA were colonized with *Aspergillus* or had unrecognized invasive infections either in the past or up to the time of HSCT or chemotherapy. Our IgM data imply that the pathogen–host interaction was not initiated in the period immediately before HSCT or chemotherapy, but rather was more long-standing. These conclusions have 2 important implications for pathogenesis. In certain patients, episodes of colonization or unrecognized infection may signal a particular susceptibility to *A fumigatus*, such as may be seen with underlying lung abnormalities or defects in innate immunity. Moreover, in the face of ongoing colonization or infection, IA may reflect progression from endogenous sources after the onset of profound immunosuppression.

The latter possibility is consistent with the observation that baseline IgG responses were predictive of early-onset IA, but not of late-onset IA. Indeed, most cases of late-onset IA are believed to be newly acquired in the community after hospital discharge. Such acquisition is in keeping with the generally accepted model for the pathogenesis of IA in HSCT recipients and patients with hematologic malignancies, in whom disease is attributed to the inhalation of a sufficient inoculum of *Aspergillus* conidia by a susceptible host [32,33]. Clearly, de novo acquisition of IA occurs in the hospital and community [34-40]. Even in outbreak situations, however, only a minority of cases of IA are conclusively linked to environmental strains [34,41], suggesting that mechanisms of pathogenesis in addition to acute inhalation may be relevant.

The major clinical implication of our findings is that baseline antibody testing against *A fumigatus* proteins may be useful as a screening test to identify patients at particularly low risk for developing IA. For example, if baseline serum IgG concentrations against enolase (the best performing of the proteins) were used for screening a population with a disease prevalence of 15%, then the anticipated PPV would be only 25% (Table 2); however, the NPV would be 95%. Moreover, 50% of the population would have a negative baseline test and fall into a low-risk group (Table 2). These values are similar to those anticipated for IgG responses against catalase based on previously published sensitivity and specificity values; the NPV of catalase also would be 95%, and 76% of patients would test negative at baseline. In populations with lower disease prevalence, NPVs would improve, and more patients would test negative. Therefore, data from separate studies suggest that IgG screening against certain *A fumigatus* proteins has high NPV for IA and would identify a significant number of low-risk patients.

Of course, determinations of whether prophylactic strategies built on such screening are worthwhile must weigh the potential benefits and costs. Our data suggest that benefits would include targeting antifungal therapy toward a subgroup that includes the vast majority of patients who are most likely to develop IA, while avoiding unnecessary antifungal exposure in a large number of low-risk patients. The costs would reflect the consequences of false-positive and false-negative test results. To continue our example, baseline anti-enolase testing of 1000 HSCT recipients in the foregoing population would allow us to administer prophylaxis to 125 patients who would be anticipated to develop IA and avoid antifungal exposure in 475 who would not develop IA. At the same time, we would fail to give prophylaxis to 25 patients with IA and give prophylaxis to 375 patients who would not develop IA. Patients in the latter group would incur unnecessary financial expenses and face the possibility of untoward events such as drug toxicity, deleterious drug–drug interactions, and emergence of antifungal resistance or selection of drug-resistant fungi.

Our small sample size limits our ability to draw definitive conclusions about the convalescent antibody data. Nevertheless, our findings indicate that increased serum IgG responses against Hsp90, Pep2, and Crf1 at week 4 compared with baseline were more common in the patients with IA who lived than in those who died. Several previous studies reported that absent or declining antibody responses against *A fumigatus* culture filtrates, a 40-kDa antigen, and recombinant proteins were associated with death from IA [25,42-44]. Based on these observations, it has been proposed that increasing antibody titers at the end of immunosuppression are indicative of recovery from IA, whereas the absence of such responses suggests a poor prognosis [15]. On the other hand, a recent prospective study did not detect increases in anti-*Aspergillus* antibody responses during the course of IA, nor did antibody responses discriminate between patients who clinically responded to antifungal therapy and those who did not [45]. In the present study, it is possible that increased IgG responses are simply markers for other determinants of survival, such as an improved overall state of health or a reconstituted immune system. At the same time, antibody responses against one or more of the proteins might have contributed to the resolution of disease. In this regard, it

### Table 3. Serum IgG Responses in Convalescent Sera, Stratified by Outcome

<table>
<thead>
<tr>
<th>Recombinant Protein</th>
<th>Lived</th>
<th>Died</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enolase</td>
<td>50 (2 of 4)</td>
<td>75 (6 of 8)</td>
<td>.54</td>
</tr>
<tr>
<td>Ahp1</td>
<td>75 (3 of 4)</td>
<td>33 (3 of 9)</td>
<td>.27</td>
</tr>
<tr>
<td>Hsp90</td>
<td>75 (3 of 4)</td>
<td>0 (0 of 9)</td>
<td>.01</td>
</tr>
<tr>
<td>Crf1</td>
<td>75 (3 of 4)</td>
<td>0 (0 of 9)</td>
<td>.01</td>
</tr>
<tr>
<td>Cdc37</td>
<td>75 (3 of 4)</td>
<td>33 (3 of 9)</td>
<td>.3</td>
</tr>
<tr>
<td>Pep2</td>
<td>100 (4 of 4)</td>
<td>11 (1 of 9)</td>
<td>.007</td>
</tr>
</tbody>
</table>
is notable that studies in a Galleria mellonella (wax moth) model showed that combination therapy with an Hsp90 inhibitor and an echinocandin rescued larvae from A fumigatus infections [46].

Future studies are indicated to verify associations between baseline IgG responses and the subsequent development of IA, and to more conclusively establish the sensitivity, specificity, and predictive values of such testing. Given that the baseline data suggest that some patients who develop IA had significant colonization or ongoing Aspergillus infections at baseline, it will be valuable to study antibody tests in conjunction with detection assays such as serum galactomannan or polymerase chain reaction. In any future studies, it will be important to establish that the utility of antibody testing is not limited by interpatient and intrapatient variability. If our baseline data are corroborated, then standardized antibody assays should be developed and validated. These assays would facilitate clinical trials of preventive strategies built on serum IgG screening. Validation of the specific correlation between baseline IgG responses and early-onset IA, for example, would provide a rationale for antifungal prophylaxis during the first 2 months after HSCT or high-risk chemotherapy. If future studies confirm that convalescent responses against particular proteins correlate with survival after IA, then the protective roles of these responses should be investigated. Finally, antibody testing of solid organ transplant recipients and other populations at high risk for IA may provide insight into unique mechanisms of pathogenesis and identify additional diagnostic and therapeutic targets and potential virulence factors. Along these lines, baseline profiling of lung transplant recipients may be particularly interesting, given our previous report that unrecognized IA and other invasive mold infections in explanted lungs are associated with posttransplantation fungal infections and poor outcomes [47].

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