The Serum Galactomannan Index Predicts Mortality in Hematopoietic Stem Cell Transplant Recipients With Invasive Aspergillosis

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We examined the relationship between serum and bronchoalveolar lavage (BAL) galactomannan index (GMI) values and mortality in allogeneic hematopoietic cell transplant recipients with invasive pulmonary aspergillosis. Using a clinical sign and symptom–initiated approach, we found that the serum but not the BAL GMI level correlated with 42- and 180-day patient mortality.

Keywords. aspergillosis; Aspergillus; mortality; galactomannan; transplant.

Invasive pulmonary aspergillosis (IPA) remains a significant cause of infectious morbidity and mortality in hematopoietic cell transplant (HCT) recipients [1]. Rapid diagnosis of IPA improves patient survival, prompting the development and implementation of fungal antigen–based diagnostic strategies in high-risk patients. Galactomannan (GM) is a polysaccharide released during Aspergillus growth at portals of infection, and is used as a biomarker of Aspergillus disease. GM detection in the circulation and in tissue reservoirs has emerged as a valuable and noninvasive adjunct to traditional culture- and histopathology-based methods to diagnose IPA. Researchers have recently shown that a decrease in serum GM indices (GMI) during early therapy correlates with favorable outcomes [2–7]. However, it is unclear if either the site (serum vs airway) or magnitude of the GMI at the time of diagnosis has prognostic significance. At our institution, the GMI is routinely tested in both serum and bronchoalveolar lavage (BAL) samples in patients with suspected aspergillosis. In this study, we examined the prognostic significance of the presence, magnitude, and location (serum vs BAL) of the GMI at the time of diagnosis on respiratory- and all-cause mortality.

METHODS

Study Design and Patient Population
We retrospectively analyzed a cohort of adult patients diagnosed with IPA between 2004 and 2010 within 100 days of their first allogeneic HCT at the Fred Hutchinson Cancer Research Center (FHCRC). GMI testing in our cohort was prompted by clinical suspicion; a surveillance strategy was not used. However, there was a standardized workup: The first clinical sign or symptom (cough, chest or pleuritic discomfort, dyspnea, hypoxemia, or fever unresponsive to broad-spectrum antibacterial antibiotics) triggered a chest computed tomography (CT) that, if abnormal, prompted a BAL with culture and GMI test and a serum GMI test. In all cases, serum GMI tests were performed within 7 days of the BAL (median 1 day prior). GMI testing was performed using the Bio-Rad Platelia Assay. A GMI of ≥0.5 with a confirmatory index processed separately on the same sample was considered positive for both serum and BAL fluid samples. The standard BAL practice was to sample the site of radiographic abnormality. Patients with radiographic or clinical evidence of extrapulmonary disease were excluded from the study. All patients provided written consent and this study was approved by the FHCRC institutional review board.

We extracted patient information on mortality, radiographic studies, transplant details, and baseline characteristics from a prospectively maintained database and chart review. We examined both all-cause and respiratory-specific mortality at prespecified times after IPA diagnosis (42 and 180 days), and the reviewer of respiratory mortality was blinded to test results. These time points were chosen based on use in previous literature. A death was considered respiratory in nature if respiratory failure was a contributing factor in the progression to death and there was chest imaging (chest radiography or CT) demonstrating an abnormal pulmonary process. If a patient had relapsed disease or severe gut graft-vs-host disease (GVHD; grades 3–4) at time of death, or if an autopsy clearly demonstrated another cause of death, the death was considered nonrespiratory.

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Diagnosis of proven or probable IPA was based on the 2008 revised European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group criteria [8]. A probable diagnosis required the presence of a host, radiographic, and mycologic factor; the latter consisted of a positive serum GMI, a positive BAL GMI, and/or growth of Aspergillus in the BAL fluid culture. Possible cases of IPA were excluded from our study.

**Statistical Analysis**

We compared clinical outcomes in patients with proven or probable IPA characterized by a serum or BAL GMI level of greater than or equal to 0.5, 1.0, 1.5, and 2.0 to patients with proven or probable IPA with a GMI level of <0.5 in the corresponding compartment.

In addition to serum and BAL GMI, we examined other risk factors based on a prior study at our institution [9]: age at transplant, sex, underlying hematologic disorder, human leukocyte antigen matched donor, myeloablative transplant, elevated creatinine (≥2.5 mg/dL) and elevated bilirubin (>6.5 mg/dL) at time of IPA diagnosis, presence of neutropenia and lymphopenia (absolute neutrophil count <500 cells/µL and absolute lymphocyte count <300 cells/µL in the 3 days prior to diagnosis of IPA, respectively), acute GVHD grade ≥3 at time of diagnosis of IPA, steroid usage (≥2 mg/kg/day prednisone), use of mold-active drugs (voriconazole, posaconazole, amphotericin derivatives, echinocandins) prior to diagnosis, and poor-risk underlying disease. Poor-risk underlying disease was based on previously reported definitions [10]. For all non-GM variables, the multivariable model included serum GMI ≥1 as the basic GM covariate as it provided the best power and discrimination.

We used Kaplan-Meier curves to estimate survival probabilities, and Cox proportional hazards models to evaluate univariate and adjusted hazard ratios (HRs) of 180-day all-cause mortality associated with the different serum and BAL GMI cutoffs. A competing risk model was used to analyze respiratory-specific mortality. We included variables in the multivariable analysis of mortality if they had a P value <.2 in univariate analysis; in the final model, statistical significance was defined as P < .05. All statistical analyses were performed using Stata statistical software, version 12 (StataCorp).

**RESULTS**

We identified 100 patients meeting the inclusion criteria with either proven (n = 3) or probable (n = 97) IPA between 2004 and 2010. Baseline patient characteristics are presented in Supplementary Table 1. Sixty-eight patients had a positive BAL GMI, 53 had a positive serum GMI, and 25 patients were positive by both tests. Eighteen patients had a positive BAL culture (15 Aspergillus fumigatus, 1 Aspergillus niger, 1 Aspergillus versicolor, 1 Aspergillus ustus); 4 of these did not test positive by GMI testing of the serum and BAL (Supplementary Table 2).

In patients with a positive serum GMI, we found significant associations between the serum GMI magnitude and 42-day respiratory, 180-day respiratory, and 180-day all-cause mortality. Figure 1A and 1B show Kaplan-Meier survival curves for patients with different serum GMI (Figure 1A) and BAL GMI (Figure 1B) cutoffs at the time of IPA diagnosis, illustrating that patients with a positive serum GMI had worse survival than those with a negative serum GMI, and that higher serum GMI levels correlated with higher patient mortality. Similarly, Figure 1C shows the cumulative incidence curves for respiratory-specific mortality by serum GMI level. In contrast, no apparent association was seen between BAL GMI and all-cause or respiratory mortality (Figure 1B and 1D). To exclude that different fungal burden among the groups could confound our results, we restricted our analysis to patients (n = 25) who tested positive by both tests and found a similar association between serum GMI levels and mortality as in the complete patient cohort (Supplementary Table 3).

Respiratory mortality at 42 days had the most pronounced survival difference in univariate and multivariable analyses: a serum GMI ≥2 had an HR of 6.6 (P = .003; Table 1). Similarly, increasing HRs of respiratory and all-cause mortality at 180 days was seen with escalating serum GMI levels: The adjusted HR for respiratory mortality increased from 2.25 with serum GMI ≥0.5 to an HR of 4.9 with serum GMI ≥2. A similar association was seen with 180-day all-cause mortality. In our multivariable models for all-cause mortality, sex, poor-risk underlying disease, acute GVHD grade ≥3, and creatinine ≥2.5 mg/dL met our prespecified univariate statistical criterion for inclusion. For respiratory-specific mortality, myeloablative transplant and creatinine ≥2.5 mg/dL were included. However, none of the covariates were statistically significant in the final model.

**DISCUSSION**

This study shows that the magnitude of the serum GMI correlates with increased respiratory-specific and all-cause mortality in allogeneic HCT recipients diagnosed with IPA. One interpretation is that higher serum GMI levels represent increased Aspergillus burden, which leads to microangiinvasion at the interface of the alveoli and blood vessels and thus to galactomannanemia. Additionally, the presence of serum GM can indicate the occurrence of micro- or macro-dissemination not captured in the workup of our patients. While it is possible that increased serum GMI could be a marker for overall poor health due to a secondary factor, we attempted to mitigate confounding by adjusting for patient and transplant characteristics that have been reported to be linked to Aspergillus-related mortality [9].
Respiratory-specific mortality showed a pronounced relationship with serum GMI levels and such events were more closely linked relative to the timing of IPA diagnosis. In contrast, the presence and magnitude of BAL GMI did not have mortality implications; this may be because it reflected a more localized infection, or an infection that the patient was able to successfully contain.

Strengths of our study were its performance at a single center, a uniform workup, a prospectively maintained database, complete clinical records, and the inclusion of only probable and proven cases. Additionally, the study was performed during the voriconazole era, so treatment was highly uniform in our patients. A limitation of our study was the potential variability in BAL volume, which was not available in this retrospective study. We plan to address this issue prospectively using the urea dilution method [11]. Additionally, BAL GMI testing is reliant on successful sampling of the radiographic pulmonary lesion(s). By nature of our inclusion criteria, patients had a high pretest probability of IPA, and the majority of IPA cases were associated with neutropenia. Therefore, our results may not apply to GMI testing performed according to surveillance strategies, or in different patient groups (eg, corticosteroid-treated allogeneic HCT patients with late IPA).

Our findings suggest that the serum GMI test provides a valuable adjunct to BAL GMI testing in patients with suspected

Table 1. Unadjusted and Adjusted Hazard Ratio for Mortality by Serum Galactomannan Index Level

<table>
<thead>
<tr>
<th>Serum GMI (≥)</th>
<th>Unadjusted HR (95% CI)</th>
<th>P Value</th>
<th>Adjusted HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>42-day respiratory mortality&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>3.30 (1.21–9.04)</td>
<td>.020</td>
<td>3.01 (1.06–8.53)</td>
<td>.038</td>
</tr>
<tr>
<td>1.0</td>
<td>4.24 (1.45–12.4)</td>
<td>.008</td>
<td>4.09 (1.33–12.5)</td>
<td>.014</td>
</tr>
<tr>
<td>1.5</td>
<td>5.22 (1.76–15.5)</td>
<td>.003</td>
<td>5.09 (1.64–15.8)</td>
<td>.005</td>
</tr>
<tr>
<td>2.0</td>
<td>6.97 (2.21–21.4)</td>
<td>.001</td>
<td>6.56 (1.88–22.9)</td>
<td>.003</td>
</tr>
<tr>
<td>180-day respiratory mortality&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>2.25 (1.07–4.72)</td>
<td>.032</td>
<td>1.86 (1.90–3.84)</td>
<td>.093</td>
</tr>
<tr>
<td>1.0</td>
<td>3.02 (1.34–6.83)</td>
<td>.008</td>
<td>2.54 (1.13–5.69)</td>
<td>.24</td>
</tr>
<tr>
<td>1.5</td>
<td>3.58 (1.54–8.34)</td>
<td>.003</td>
<td>2.96 (1.26–6.98)</td>
<td>.013</td>
</tr>
<tr>
<td>2.0</td>
<td>4.90 (1.92–13.0)</td>
<td>.001</td>
<td>4.01 (1.58–10.1)</td>
<td>.003</td>
</tr>
<tr>
<td>180-day all-cause mortality&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>1.68 (1.96–2.95)</td>
<td>.69</td>
<td>1.75 (1.99–3.09)</td>
<td>.56</td>
</tr>
<tr>
<td>1.0</td>
<td>2.18 (1.15–4.15)</td>
<td>.017</td>
<td>2.12 (1.10–4.06)</td>
<td>.24</td>
</tr>
<tr>
<td>1.5</td>
<td>2.97 (1.53–5.81)</td>
<td>.001</td>
<td>2.95 (1.47–5.91)</td>
<td>.002</td>
</tr>
<tr>
<td>2.0</td>
<td>3.68 (1.63–8.30)</td>
<td>.002</td>
<td>4.08 (1.68–9.87)</td>
<td>.002</td>
</tr>
</tbody>
</table>

Serum galactomannan index reference is <0.5.
Abbreviations: CI, confidence interval; GMI, galactomannan index; HR, hazard ratio.
<sup>a</sup> Multivariable model adjusted for myeloablative transplant and creatinine ≥2.5 mg/dL.
<sup>b</sup> Multivariable model adjusted for sex, poor-risk underlying disease, acute graft-vs-host disease, and elevated creatinine.

Respiratory-specific mortality showed a pronounced relationship with serum GMI levels and such events were more closely linked relative to the timing of IPA diagnosis. In contrast, the presence and magnitude of BAL GMI did not have mortality implications; this may be because it reflected a more localized infection, or an infection that the patient was able to successfully contain.

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IPA. GMI seropositivity is important for early recognition of IPA patients at risk for a poor outcome. Additionally, serum GMI seropositivity could be used as an important covariate in future analyses on outcomes in these patients or as a stratification variable in randomized trials.

We conclude that the serum GMI level, at the time of IPA diagnosis, is an important predictor of mortality in allogeneic HCT recipients. These data have implications for prognosis, monitoring, and study design.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. M. B. has served as a consultant to Astella and Merck and has received research support from Merck. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References