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Galactomannan Testing in Bronchoalveolar Lavage Fluid Facilitates the Diagnosis of Invasive Pulmonary Aspergillosis in Patients with Hematologic Malignancies and Stem Cell Transplant Recipients

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Invasive pulmonary aspergillosis (IPA) is a major cause of mortality in patients with stem cell transplants and hematologic malignancies. Timely diagnosis of IPA improves survival but is difficult to make. We evaluated the effectiveness of bronchoalveolar lavage (BAL) galactomannan (GM) in diagnosing IPA in these populations by retrospectively reviewing records of 67 consecutive patients, in whom 89 BAL GM tests were performed. For patients with IPA, only the first BAL sample linked to the IPA episode was analyzed. Eighty samples were associated with proven, 12 with probable, and 32 with possible invasive fungal infections (IFI), and 37 were associated with no IFI. Among patients with IFIs, 4 had proven, 11 probable, and 32 possible IPA. Using BAL GM $\geq$0.5 (cutoff for serum GM) and $\geq$0.85 (optimal cutoff identified by receiver-operating characteristic curve), the sensitivity in diagnosing proven or probable IPA was 73% (11/15) and 67% (10/15), respectively, and specificity was 89% (33/37) and 95% (35/37). At these cutoffs, positive and negative predictive values were 73% (11/15) and 83% (10/12), and 89% (33/37) and 87% (35/40), respectively. BAL GM was more sensitive than cytology (0%, 0/14), BAL culture (27%, 4/15), transbronchial biopsy (40%, 2/5), or serum GM (67%, 10/15) for diagnosing IPA. BAL GM was $\geq$0.85 and $\geq$0.5 in 86% (6/7) and 100% (7/7) of patients with proven or probable IPA who received a mold-active agent for $\leq$3 days. BAL GM added sensitivity to serum GM and other means of diagnosing IPA, and was not impacted by short courses of mold-active agents.

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INTRODUCTION

Invasive pulmonary aspergillosis (IPA) and other types of invasive fungal infections (IFI) are major causes of mortality and morbidity among hematopoietic stem cell transplant (HSCT) recipients, patients with hematologic malignancies, and solid-organ transplant recipients. The incidence of IPA is approximately 15% to 20% among HSCT recipients and neutropenic patients with hematologic malignancies [1-4], and case fatality rates are as high as 50% to 90% despite antifungal therapy [1,3-6]. Timely diagnosis of IPA improves survival [7,8], but it is difficult to make because of the limitations of current diagnostic tests. Cultures and bronchoscopy-based techniques such as cytology and histopathology of tissue biopsies, for example, are limited by poor sensitivity and specificity, and complications of invasive procedures [9-11]. More recently, detection of galactomannan (GM), a cell wall polysaccharide of most *Aspergillus* and *Penicillium* species that is released into the serum during growth in tissue, has emerged as a useful adjunct to traditional diagnostic tests [12]. Considered along with other tests and radiographic and clinical findings, serum GM clearly improves the ability to diagnose IPA [13]. Nevertheless, the sensitivity of the test among HSCT recipients and patients with hematologic malignancies is only moderate, in the range of 61% to 71% by recent meta-analysis [14]. Moreover, the positive predictive value (PPV) of serum GM is limited by the relative infrequency of IPA, even among high-risk populations.

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Several studies have suggested that the sensitivity of GM detection in diagnosing IPA can be improved by applying the test to bronchoalveolar lavage (BAL) fluid collected during bronchoscopy. Among HSCT recipients and patients with hematologic malignancies, detection of BAL GM added to the diagnostic utility of serum GM [15-22] and sensitivity and specificity ranged from 58% to 100% and 94% to 100%, respectively [16,18,23,24]. Despite these observations, the role of BAL GM testing remains to be defined, and at least 1 study reported high rates of false-positive results [25].

We previously reported our experience with BAL GM in diagnosing IPA among solid-organ transplant recipients and nonimmunosuppressed hosts [26,27]. In this study, we review our experience with BAL GM testing among patients admitted to the hematologic malignancy or HSCT inpatient services at the Shands Hospital at the University of Florida, as the test was used by clinicians caring for these patients. Our objective was to determine the performance of BAL GM in diagnosing IPA.

METHODS

Patients with acute leukemia receiving chemotherapy or undergoing HSCT were eligible for this study, which was approved by, and performed according to the guidelines of the institutional review board at the University of Florida. All patients received fluconazole prophylaxis and had serum GM performed twice weekly. Neutropenic fever was treated with cefepime, and antibiotics were changed depending on culture results. Patients with persistent neutropenic fever despite antibiotics underwent CT scans of the chest and sinuses if sinopulmonary signs or symptoms occurred. Those with new radiologic abnormalities, positive serum GM, or sinopulmonary signs or symptoms suggestive of IFI were presumptively treated with voriconazole while additional diagnostic assessment took place. The routine voriconazole dose used at our center was 6 mg/kg per os every 12 hours for 2 doses, followed by 4 mg/kg per os twice a day. Bronchoscopy was performed if pulmonary abnormalities were detected radiologically. If zygomycosis was suspected or there was a clinical reason that voriconazole was contraindicated, amphotericin B lipid complex at 5 mg/kg intravenously was substituted.

Between September 2004 and December 2006, BAL fluid collected for diagnostic purposes underwent GM testing. GM testing was performed on all BAL specimens at MiraVista Diagnostics (Indianapolis, IN) using the Platelia *Aspergillus* enzyme immunoassay (Bio-Rad Laboratories, Redmond, WA), as described elsewhere [26,27]. The BAL GM results were made available to the patients' physicians, who made all decisions regarding management. Although the interpretive cutoff values for positive BAL GM have not been established, we adopted the 0.5 cutoff proposed for serum testing. Among patients with IPA, only the first BAL sample associated with the IPA episode was included in the evaluation.

DEFINITIONS

IFI was classified into proven, probable, and possible using the European Organization for Research and Treatment of Cancer-Mycoses Study Group [28]. IFI was further subcategorized as proven or probable IPA if the disease involved the lungs plus either culture grew *Aspergillus* spp. or serum GM index was $\geq$0.5. Detection of GM in BAL fluid was not included in the classification of IPA in this study. Classifications were made by 5 investigators who were blinded to BAL GM results at the time (M.H.N., H.L., C.J.C., L.J.W., and J.R.W.). In the event of disagreements, consensus was reached among the investigators.

Data Analysis

The sensitivity, specificity, PPV, negative predictive value (NPV), and likelihood ratio for BAL GM testing were calculated on a per-patient basis. The optimal cutoff for BAL GM was determined by receiver-operating characteristic (ROC) analysis. Univariate analysis of contingency data was done by chi-square or Fisher's exact test. Continuous variables were compared using Mann-Whitney test. P values $\leq$.05 were considered to be significant.

RESULTS

BAL Samples and GM Indices

A total of 107 BAL samples from 67 unique patients were submitted for determination of GM index. Eighty-nine samples from the 67 patients were sent for diagnostic purposes, and 18 samples from 12 patients were sent as follow-up during treatment for IFI including IPA. Among the 89 diagnostic samples, 52 were associated with proven (n = 8), probable (n = 12) or possible (n = 32) IFI, and 37 were not associated with IFI. The distribution of GM indices according to IFI classification is presented in Figure 1.

Patient Demographics and Clinical Data

The median age of patients was 63 years, and 73% (49/67) were men. Fifty-five percent (37/67) of patients received chemotherapy for hematologic malignancies (acute myelogenous leukemia (AML) = 16, acute lymphocytic leukemia (ALL) = 8, multiple myeloma = 3, others = 10), and 45% (30/67) underwent HSCT (allogeneic = 29, autologous = 1). Thirty