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The utility of bronchoalveolar lavage beta-D-glucan testing for the diagnosis of invasive fungal infections

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Summary *Objectives:* To investigate the utility of beta-D-glucan (BDG) testing in bronchoalveolar lavage (BAL) fluid for the diagnosis of invasive fungal infection (IFI), as compared to BAL galactomannan (GM).
Methods: We retrospectively reviewed medical records of 132 consecutive patients at the National Institutes of Health (NIH) in whom BAL BDG testing was performed for diagnosis of pneumonia. Using the European Organization for Research and Treatment of Cancer/Mycoses Study Group guidelines, we determined which patients had proven or probable IFI, and assessed the diagnostic performance of BAL BDG testing, relative to BAL GM. We also determined the reproducibility of the BDG assay in BAL via repeat testing of patient samples.
Results: Ten patients had *Pneumocystis* pneumonia, and 34 patients had proven/probable IFI, including 14 with invasive aspergillosis (IA). BAL BDG was 100% sensitive for *Pneumocystis*. Although BAL BDG had similar sensitivity to BAL GM for the diagnosis of IA and IFI, it exhibited

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inferior specificity. Repeat testing demonstrated poor reproducibility of the BDG assay in BAL but not in serum.
Conclusions: BDG testing exhibits poor specificity and reproducibility in BAL. Identification of the BAL-specific factors that may interfere with the performance of the assay could improve the clinical usefulness of BAL BDG testing.
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Introduction

Despite advances in antifungal therapies, invasive fungal infections (IFI), such as invasive aspergillosis (IA) and non-*Aspergillus* mold infections, continue to cause substantial morbidity and mortality in immunosuppressed patients, in part due to suboptimal diagnosis.[1] The sensitivity of respiratory cultures is unacceptably low, even in histopathology-proven cases, and invasive procedures that have a higher yield are infrequently performed due to patient comorbidities.[1,2] Molecular testing has shown promise but its implementation has been hampered by lack of standardization and non-specific results caused by colonization and/or contamination.[1,3]

In recent years, beta-D-glucan (BDG) and galactomannan (GM) tests, which detect fungal cell wall constituents, have emerged as indirect diagnostic markers for IFI. These tests have shown variable sensitivity and specificity depending on several clinical factors. For example, both BDG and GM are more sensitive in patients with neutropenia and/or hematologic malignancies compared to solid organ transplant recipients.[4,5] False-positive results have been reported with concurrent bacterial infections (BDG) or antibiotic administration (GM), and false-negative results for both tests have been associated with antifungal prophylaxis.[1,6] The site of testing also seems to play a role as shown by the higher sensitivity of bronchoalveolar lavage (BAL) over serum GM for diagnosing IA.[7] While serum BDG is an approved test by the Food and Drug Administration and may aid in the diagnosis of IA and non-*Aspergillus* fungal infections, which are less amenable to detection by GM,[1] the role of BDG testing in the BAL for the diagnosis of IFI remains unclear. Thus, our study aimed to examine the performance of BAL BDG (relative to BAL GM) in an unselected population of patients at high-risk for IFI.

Patients and methods

We reviewed medical records of 132 consecutive patients seen at the National Institutes of Health (NIH) between January 2008 and December 2011 for whom BAL BDG was performed for work-up of suspected fungal pneumonia. We obtained exemption from Institutional Review Board review by the NIH Office of Human Subjects Research Protections and collected information on demographics, underlying diseases/predisposing factors, clinical/radiographic features, microbiologic data and clinical outcome. When available, values for BAL GM ($n = 129$), serum BDG ($n = 76$) and serum GM ($n = 73$) were also recorded. Samples were tested in real-time after collection of BAL/serum from patients. The Fungitell® assay (Beacon Diagnostics Laboratory, East Falmouth, MA) was used for BDG testing, and the Platelia™ *Aspergillus* enzyme immunoassay (Bio-Rad, Redmond, WA) was used for GM testing. BDG testing was performed in triplicate from all samples. Per manufacturer recommendations, GM testing was performed in duplicate from all positive samples. For both serum and BAL, BDG values of $\geq$80 pg/mL and $<$80 pg/mL were considered positive and negative, respectively, and a GM index of $\geq$0.5 and $<$0.5 was considered positive and negative, respectively.

Cases of proven/probable IFI or IA, and their response to treatment, were defined per European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) guidelines.[3,8] Accordingly, *Pneumocystis* pneumonia (PCP) cases, defined as positive *Pneumocystis* polymerase chain reaction and/or direct fluorescent antibody, with compatible clinical picture, were not grouped within IFI, but analyzed separately.

We determined the sensitivity and specificity of each test (BAL BDG, BAL GM, serum BDG and serum GM) for all IFI, and for IA and PCP separately. We also examined the impact of pre-exposure to antibacterials/antifungals, fungal colonization, and concomitant infections on test performance.

To interrogate the reproducibility of BDG testing in BAL, 17 randomly selected BAL patient samples underwent repeat testing by the same operator at the NIH Microbiology Laboratory or the assay reference laboratory (Beacon Diagnostics) and were evaluated for reproducibility by means of the coefficient of variation (CV). Twenty-two randomly selected serum patient samples also underwent repeat testing as a control to determine the site-specific reproducibility of the BDG assay. For both BAL and serum samples that underwent repeat testing, reproducibility testing was performed on frozen specimens, which were thawed only once. All work was performed in a biological safety cabinet with careful consideration to prevent contamination. Technologists performing the testing were fully trained and had passed proficiency testing for the BDG assay. Per manufacturer guidelines for serum BDG testing, a maximum CV of 20% is expected for replicates of samples with BDG values between 60 and 500 pg/mL. The percent of repeat test values for BAL and serum samples that fell within the expected CV was recorded.

Statistical analysis was performed using SAS, Version 9.3 (SAS Institute Inc., Cary, NC, 2011). The sensitivity and specificity of each test (BAL BDG, BAL GM, serum BDG, serum GM) for the diagnosis of all IFI, IA and PCP was determined. Receiver operating characteristic (ROC) area under the curve (AUC) analysis was used to evaluate whether test values were predictive of IFI. Among those with a positive test, logistic regression was used to assess whether the magnitude of positive test values was predictive of mortality. Fisher's exact test was used to identify associations between clinical factors and false positive or false negative results. For all analyses, $P < 0.05$ was considered statistically significant.

Results

The most common host factors in our cohort were hematologic malignancy ($n = 58$), allogeneic hematopoietic stem cell transplantation ($n = 40$), neutropenia ($n = 31$) and/or corticosteroid use ($n = 30$). The majority of patients ($n = 78$) were on prophylactic or empiric antifungal treatment with fluconazole ($n = 37$), voriconazole ($n = 33$) and/or caspofungin ($n = 14$) prior to BAL; some patients received more than one antifungal agent (Table 1).