



Performance of the (1,3)-Beta-D-Glucan Assay on Bronchoalveolar Lavage Fluid for the Diagnosis of Invasive Pulmonary Aspergillosis

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Abstract Detection of (1,3)-beta-D-glucan (BDG), a component of the cell wall of many fungi, was studied in bronchoalveolar lavage fluid (BALF) as a possible aid for the diagnosis of proven/probable invasive pulmonary aspergillosis (IPA). BDG was measured on stored BALF from 13 patients with EORTC/MSGERC defined proven/probable IPA and 26 matched control patients without IPA. The median BALF BDG was 80 pg/mL (range < 45–8240 pg/mL) in the IPA cohort and 148 pg/mL (range

< 45–5460 pg/mL) in the non-IPA cohort. Using a positive cutoff of ≥ 80 pg/mL, sensitivity was 54% and specificity was 38%. Higher cutoff values led to improvement in specificity but a dramatic decrease in sensitivity. ROC/AUC analysis was unable to identify an optimal cutoff value at which test performance was enhanced: AUC 0.43, 95% CI 0.24–0.63. When the BDG assay was performed on BALF, neither sensitivity nor specificity was sufficient for use in the diagnosis of IPA.

Keywords Aspergillosis · Pneumonia · Beta-D-glucan · Bronchoalveolar lavage

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Introduction

Non-culture-based methods have become an important tool for the diagnosis of invasive pulmonary aspergillosis (IPA), as culture-based methods cannot reliably identify invasive pulmonary infection [1]. Detection of (1,3)-beta-D-glucan (BDG), a component of the cell wall of many fungi, in serum has been reported to have sensitivity as high as 94–96% for the diagnosis of *Pneumocystis jirovecii* pneumonia and 85% for invasive candidiasis [2–4], but is less sensitive for the diagnosis of IPA [5, 6]. Bronchoalveolar lavage fluid (BALF) is frequently obtained from patients with IPA and is often used for other non-

culture-based testing methodologies, including *Aspergillus* galactomannan (GM) testing. We sought to determine whether the BDG assay in BALF might prove useful in the diagnosis of IPA.

Materials and Methods

This retrospective, single-center study was performed at the University of Michigan Health System and was approved by the Institutional Review Board. Residual BALF samples from bronchoscopy procedures performed for clinical care were collected from 11/2015 to 7/2019 and stored at -70°C . The study population consisted of patients who had proven or probable IPA defined by 2019 EORTC/MSGERC consensus definitions [7]. Patients were matched 1:2 by gender, age within 5 years, and risk factors for IPA with control patients who did not have IPA. Patients without host factors or clinical features for IPA, but with *Aspergillus* identified on BALF cultures, were excluded from the control group. Clinical data were collected from the electronic medical record and entered into a REDCap® database. BDG testing was performed on coded BALF samples at Viracor Eurofins Laboratory (Lee's Summit, MO) using the Fungitell® assay (Associates of Cape Cod, MA).

Sensitivity and specificity were determined for BDG testing of BALF. Receiver operating characteristic/area under the curve (ROC/AUC) analysis was used to determine an optimal cutoff value of BALF BDG that might be predictive for IPA. Statistical analysis was performed using SAS 9.4 statistical software.

Results

The IPA cohort consisted of 13 patients, 11 of whom had probable and 2 of whom had proven pulmonary aspergillosis. The mean age of the 8 men and 5 women was 51.3 ± 18 years. Risk factors for IPA included lung transplant ($n = 5$), hematological malignancy ($n = 4$), T cell immunosuppressive agents ($n = 5$), high-dose corticosteroids ($n = 2$), critical illness ($n = 2$), Cushing's disease ($n = 1$), and HIV infection with $\text{CD4} < 200$ cells/ μl ($n = 1$). The matched control cohort of 26 patients included 16 men and 10 women with mean age 51.6 ± 18.4 years. Host factors included

solid organ transplant ($n = 8$), hematological malignancy ($n = 7$), high-dose corticosteroids ($n = 3$), T cell immunosuppressive agents ($n = 3$), critical illness ($n = 3$), chronic obstructive pulmonary disease ($n = 3$), diabetes ($n = 3$), HIV infection with $\text{CD4} < 200$ cells/ μl ($n = 1$), and autoimmune disease requiring immunosuppression ($n = 1$). Mycological evidence of IPA in the case cohort included positive BALF culture ($n = 7$), BALF GM > 1.0 ($n = 5$), serum GM > 1.0 ($n = 1$); two patients had proof of IPA at autopsy (Table 1).

The median and range of BALF BDG values were 80 pg/mL (range < 45 –8240 pg/mL) in the IPA cohort and 148 pg/mL (range < 45 –5460 pg/mL) in the control cohort (Fig. 1). Using a cutoff value of ≥ 80 pg/mL, the sensitivity and specificity were 54% and 38%, respectively. Higher cutoff values revealed improvement in specificity but a dramatic decrease in sensitivity. ROC/AUC analysis failed to identify an optimal cutoff value at which the test performance was enhanced. AUC was poor: 0.43, 95% CI 0.24–0.63. Higher BALF BDG levels did not predict increased likelihood of IPA.

Discussion

We evaluated the performance of the BDG assay on BALF as a possible aid for the diagnosis of IPA by comparing a cohort with well-characterized IPA with a matched control cohort. The BALF BDG assay had poor sensitivity and specificity for the diagnosis of IPA when a population of patients with diverse risk factors for IPA were studied.

Other studies have evaluated BALF BDG for the diagnosis of IFI; [8–12] fewer studies focused specifically on IPA [13, 14]. Studies in solid organ transplant recipients found that BDG testing on BALF was moderately sensitive (79%) for IFI [8], but another study specifically of IPA found a sensitivity of only 53% [13]. Specificity varied from 39 to 70% [8, 13]. Other studies assessed primarily patients with hematological diseases and noted that sensitivity of BALF BDG for IFI was 73% and for IPA was 71%, but specificity was only 48–53% [9, 10]. Evaluations of BALF BDG in populations with diverse risk factors for IFI and IPA, similar to our population, found in one study, sensitivity of 50% and specificity of 55% for the diagnosis of IFI [11], but in another, sensitivity was

Table 1 Demographics, underlying immunosuppression, and test results for 13 patients who underwent bronchoalveolar lavage (BAL) and were diagnosed with invasive pulmonary aspergillosis

Age/gender comorbidity	Aspergillosis category	Antifungal prophylaxis ^a	GM BALF (ODI)	GM Serum (ODI)	BDG BALF (pg/mL)	BDG Serum (pg/mL)	BALF culture	CT chest findings
32/F SLE, steroids ^b	Proven at autopsy	No	< 0.5	NA	< 45	387	<i>A. fumigatus</i>	Pulmonary nodules
42/M Cushing's D., critical illness	Proven at autopsy	No	< 0.5	< 0.5	91	< 31	No growth	GGO, pulmonary nodules
67/M Lung Tx	Probable	No	6.7	< 0.5	164	94	<i>A. fumigatus</i>	Pulmonary nodules
22/M AML	Probable	FLU	6.2	< 0.5	80	< 31	No growth	Pulmonary nodules
42/M Lung Tx	Probable	No	< 0.5	NA	52	NA	<i>A. fumigatus</i>	Pulmonary nodules
62/M Lung Tx	Probable	No	< 0.5	NA	< 45	NA	<i>A. fumigatus</i>	GGO, diffuse pulmonary infiltrates
23/F Lung Tx	Probable	No	< 0.5	< 0.5	< 45	2240	<i>A. fumigatus</i>	Pulmonary nodules
66/M CMML	Probable	No	< 0.5	1.75	131	< 31	No growth	GGO, pulmonary nodules
79/F CLL	Probable	No	NA	< 0.5	430	< 31	<i>A. fumigatus</i>	Pulmonary nodules
58/F steroids ^b , critical illness	Probable	No	7.6	NA	8240	< 31	NA	Pulmonary nodules
57/M HIV with CD4 < 200	Probable	No	7.4	<0.5	75	99	No growth	Consolidation, pulmonary nodules
49/F Lung Tx	Probable ^c	ISA	NA	NA	<45	NA	<i>A. fumigatus</i>	No pulmonary changes
68/M MDS	Probable	No	2.43	< 0.5	700	< 31	No growth	Cavitary nodules, consolidation, GGO

AML acute myeloid leukemia, BALF bronchoalveolar lavage fluid, BDG beta-D-glucan, CLL chronic lymphocytic leukemia, CMML chronic myelomonocytic leukemia, D disease, FLU fluconazole, GM galactomannan, GGO ground glass opacities, ISA isavuconazole, MDS myelodysplastic syndrome, NA not available, ODI optical density index, SLE systemic lupus erythematosus, Tx transplant

^aProphylaxis at the time bronchoalveolar lavage was performed

^bHigh-dose corticosteroids equivalent to prednisone dose ≥ 0.3 mg/kg daily for at least 3 weeks

^cTracheobronchial anastomotic infection

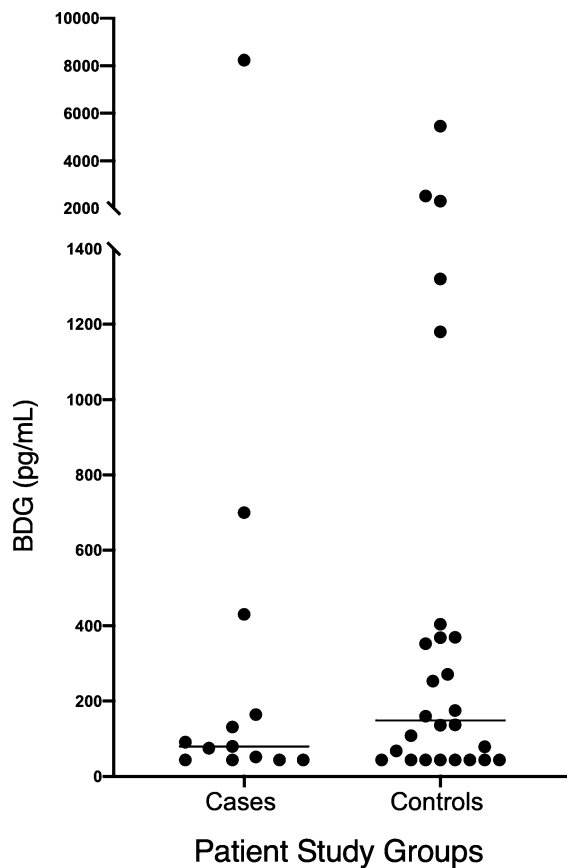


Fig. 1 Bronchoalveolar lavage fluid (BALF) beta-D-glucan (BDG) concentrations expressed as pg/mL in 13 patients with invasive pulmonary aspergillosis (IPA) and 26 matched control patients. Bars represent the median BDG level for that cohort

71–88% and specificity 73–84% for IPA [14]. Meta-analysis of the available studies noted great heterogeneity and overall sensitivity and specificity of 52% and 58%, respectively [12].

We found specificity was particularly limited when the BDG assay was performed on BALF. Higher specificity has been noted with measurement of BDG in serum than in BALF [9–11]. The reasons for increased false positive BDG results when testing BALF are not known, but could be related to the fluid used for lavage or the presence of colonizing organisms, including *Candida* species and Gram-negative bacilli, in the lower respiratory tract.

Limitations of our study include its single-center, retrospective design and the relatively small number of IPA cases studied. We were not able to control when bronchoscopy was performed in the course of

the patient's pulmonary disease, which could influence the sensitivity of the assay. The impact of mold active antifungal exposure on BDG assay performance at time of bronchoscopy is uncertain, but in our study, only one patient was on a mold active agent at the time BALF was collected.

Our study found that sensitivity and specificity of the BDG assay on BALF were poor, and our results do not support the use of the BDG test on BALF for the diagnosis of IPA.

Author contributions Kathleen A. Linder, MD was involved in investigation, resources, data curation, and writing original draft. Carol A. Kauffman, MD contributed to resources, visualization, writing—review and editing. Shiwei Zhou, MD helped in resources and data curation. Blair J. Richards, MPH helped in formal analysis. Steve Kleiboeker, PhD was involved in resources and validation. Marisa H. Miceli, MD helped in conceptualization, methodology, investigation, supervision, project administration, visualization, writing—review and editing.

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Compliance with ethical standards

Conflict of interest All authors have no conflicts of interest related to this study.

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