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Beta-D-Glucan Testing For Diagnosis of Endemic Mycoses

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Antigen detection by enzyme immunoassay (EIA) is widely used for diagnosis of histoplasmosis and blastomycosis, but less often for coccidioidomycosis. The sensitivity of the MVista® antigen EIAs for *Histoplasma* in disseminated histoplasmosis was 91.6% [1], *Blastomyces* for blastomycosis 89.9% [2] and *Coccidioides* for coccidioidomycosis 57% [3]. (1→3)-β-D-glucan (BDG), a broadly reactive fungal marker, is most often used as an aid in diagnosis of candidiasis, aspergillosis, *Pneumocystis* pneumonia and uncommon mold infections.

Publications on the use of BDG detection as an aid to diagnosis of endemic mycoses are scant [4-6]. Though BDG detection is commonly used as an aid to diagnose endemic mycoses, more information on their performance is needed. Furthermore, as the test is broadly reactive and BDG can be detected in the serum of patients without fungal diseases, other tests would be needed to determine the cause of the infection.

Understanding of the less common causes for elevated BDG is required to use the test for diagnosis of fungal diseases. Literature will be reviewed, and new results will be presented in patients with histoplasmosis, blastomycosis, and coccidioidomycosis.

BDG has been used as an aid for diagnosis of endemic mycoses, though publications on its performance for this use are scant.

Methods

Specimens were selected from patients with positive *Histoplasma*, *Blastomyces*, and *Coccidioides* antigen results ranging from weakly to strongly positive that had been stored frozen at -20°C at MiraVista Diagnostics. The blastomycosis and coccidioidomycosis cases were all from the endemic areas of the United States. Five histoplasmosis cases were from endemic areas and 15 were from large reference laboratories throughout the US, although exact locations of the patients were unavailable, multiple specimens were submitted for *Histoplasma* antigen detection and were positive.

The BDG test was performed according to the package insert of the manufacturer. Results > 80 pg/mL were considered positive. Results < 80 pg/mL were considered negative, including those between 60 and 79 pg/mL which were indeterminate and considered negative.

Commercial software was used for statistical analysis (Sigma Plot 14.0, Systat Software). Shapiro-Wilk test was used to test for normality and data was non-parametric. Descriptive statistics were provided as median and range. Positive percent agreement was calculated for each serum set (blastomycosis, coccidioidomycosis, histoplasmosis). Spearman correlation coefficients were used to describe the relationship between fungal galactomannan (GM) and BDG concentrations. Mann-Whitney U test was used to compare median BDG concentrations between the 3 serum sets. Statistical significance was set at P<0.05.



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Results

Descriptive statistics are provided in Table 1. Positive agreement between BDG and *Coccidioides* antigen positivity was 67%, *Histoplasma* antigen positivity 55% and *Blastomyces* antigen positivity 35%. The BDG concentration correlates with *Coccidioides* antigen concentration ($R=0.79$, $P<0.001$) and the *Histoplasma* antigen concentration ($R=0.54$, $P=0.013$) but not the *Blastomyces* antigen concentration ($R=0.41$, $P=0.07$) Figures 1-3). Median BDG concentrations were significantly higher in the coccidioidomycosis samples as compared with the blastomycosis samples (145 vs 46, $P=0.033$). There was not a significant difference between BDG concentrations in coccidioidomycosis samples compared with histoplasmosis samples ($P=0.57$) or the blastomycosis samples compared with histoplasmosis samples ($P=0.10$).

Positive agreement:
BDG and Cocci Ag: 67%
BDG and Histo Ag: 55%
BDG and Blasto Ag: 35%

Discussion

Others have reported on BDG detection in patients with histoplasmosis and coccidioidomycosis. BDG was detected in the serum in 5 of 6 patients with disseminated histoplasmosis in one study [4] and in 6 of 6 in another [7]. Another report of a single patient with *Histoplasma* meningitis noted a BDG level of 110 pg/mL in cerebrospinal fluid (CSF) and 14 pg/mL in serum [8] and a third report described a level of 282 pg/mL in CSF and 33 pg/mL in serum [9]. Although we observed a positive correlation between BDG concentration and *Histoplasma* antigen concentration, there was lack of correlation between high BDG levels > 80 pg/mL and positive *Histoplasma* antigen results; BDG was positive in only 55% of specimens with positive antigen results.

In one small study, BDG was detected in serum from 11 of 12 (92%) patients with coccidioidomycosis and elevated levels of *Coccidioides* antigen in serum [10]. In a study of 188 patients with positive results in serum for anti-*Coccidioides* antibodies, sensitivity for BDG detection was 44% and specificity was 91% [6]. Two studies reported BDG in CSF. The first described a patient with probable disseminated coccidioidomycosis with a BDG level of 139 mcg/mL in CSF and 14 pg/mL in serum [8]. Another study that considered results of > 31 pg/mL positive reported a sensitivity of 96% and specificity of 82% in CSF from patients with *Coccidioides* meningitis but results in serum were not reported [11]. We observed positive agreement between BDG and *Coccidioides* antigen positivity in 67% of specimens containing *Coccidioides* antigen. BDG and antigen concentration correlated significantly in serum from patients with positive *Coccidioides* antigen results.

There are no publications reporting BDG results in patients with blastomycosis, but the manufacturer reports negative results in 4 patients with blastomycosis [4]. We observed BDG concentrations > 80 pg/mL in 35% of patients with blastomycosis but BDG and antigen concentrations were not significantly correlated.

Of note is that BDG detection is not specific for fungal infection.

The manufacturer indicates that false-positive results may occur in the following situations: (1) hemodialysis, (2) receipt of fractionated blood products such as albumin and immunoglobulins, (3) exposure to glucan-contaminated gauze and surgical sponges during surgical procedures. Others have reported elevated BDG levels in serum in patients with burn injuries [12], infants receiving blood product transfusions [13] and patients receiving gamma-globulin reagents intravenously [14]. False-positive results complicate the use of BDG detection for diagnosis of *Histoplasma* meningitis [5].



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Our study has several limitations. Most important, clinical information was not available for the specimens that we tested: Studies correlating BDG performance with clinical findings are needed. However, specimens were obtained from patients residing in the endemic areas for those mycoses. The histoplasmosis specimens were chosen from patients with repeatedly positive *Histoplasma* antigen results in urine and serum, suggesting their physicians considered them to have histoplasmosis. Second, we did not assess negative agreement and the potential for false positive BDG results. Third, the number of specimens was small.

In summary, although this study increases our knowledge of the potential role for BDG detection for diagnosis of endemic mycoses, BDG's limitations must be recognized, and additional studies are needed in patients in whom the clinical findings are known.

If positive BDG results provide the only basis for diagnosis, antigen and antibody tests should be performed to strengthen the diagnosis.

Table 1. Descriptive statistics for Beta-D-glucan and galactomannan concentrations in serum samples with detectable fungal antigen.

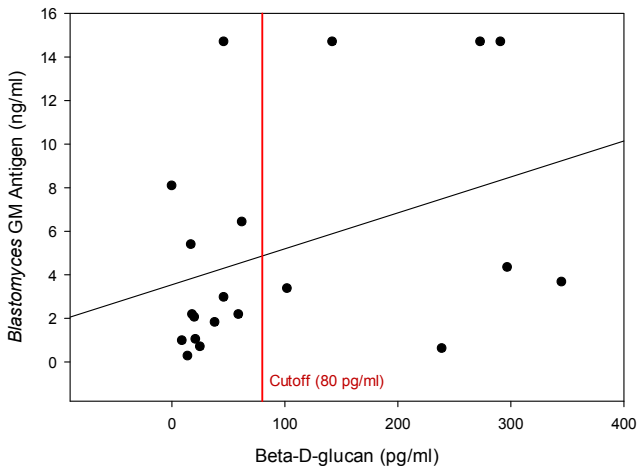
Organism	Test	Units	Medium	Minimum	Maximum
<i>Blastomyces</i>	GM	ng/ml	3.17	0.27	14.70
	BDG	pg/ml	46	0	345
<i>Coccidioides</i>	GM	ng/ml	0.50	0.11	8.20
	BDG	pg/ml	145	20	1376
<i>Histoplasma</i>	GM	ng/ml	15.60	3.30	19.00
	BDG	pg/ml	94	16	1705

GM, galactomannan; BDG, Beta-D-glucan



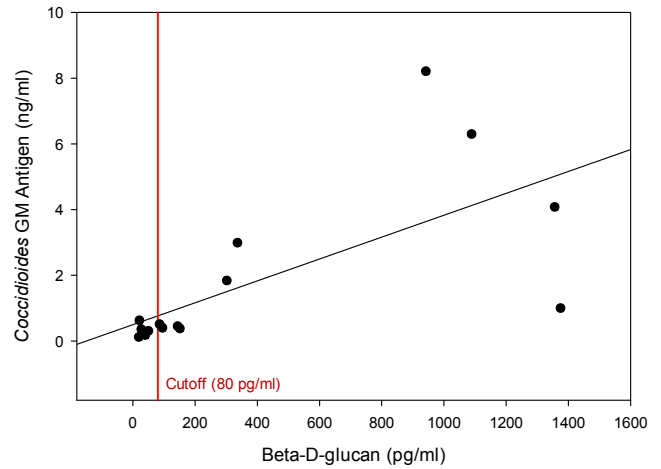
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Figure 1. Beta-D-glucan concentrations in 20 serum samples with detectable *Blastomyces* galactomannan antigen.



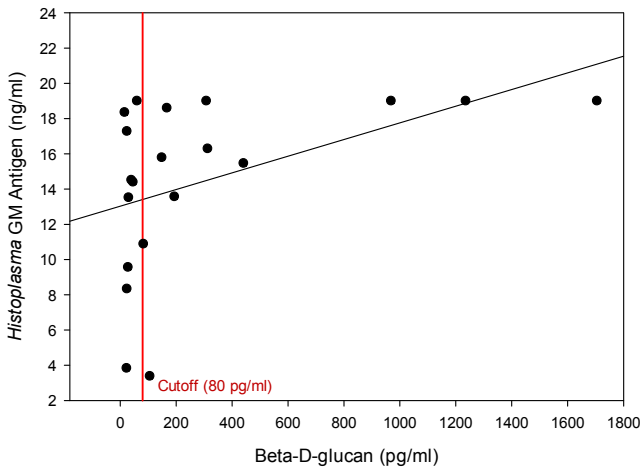
GM, galactomannan

Figure 2. Beta-D-glucan concentrations in 15 serum samples with detectable *Coccidioides* galactomannan antigen.



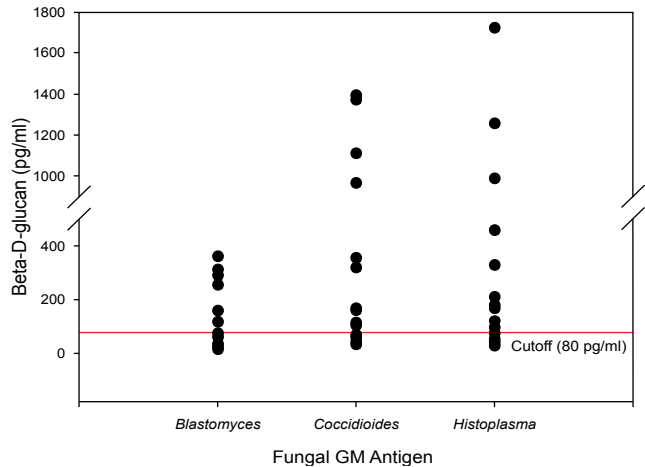
GM, galactomannan

Figure 3. Beta-D-glucan concentrations in 20 serum samples with detectable *Histoplasma* galactomannan antigen.



GM, galactomannan

Figure 4. Beta-D-glucan concentrations in serum samples that were positive for *Histoplasma*, *Blastomyces*, or *Coccidioides* antigen.



GM, galactomannan



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Reference List

1. Hage CA, Ribes JA, Wengenack NL, et al. A multicenter evaluation of tests for diagnosis of histoplasmosis. *Clin Infect Dis* 2011 Sep; 53(5):448-54.
2. Connolly P, Hage CA, Bariola JR, et al. *Blastomyces dermatitidis* Antigen Detection by Quantitative Enzyme Immunoassay. *Clin Vaccine Immunol* 2012 Jan; 19(1):53-6.
3. Kassis C, Durkin M, Holbrook E, Myers R, Wheat L. Advances in Diagnosis of Progressive Pulmonary and Disseminated Coccidioidomycosis. *Clin Infect Dis* 2020 Feb 28.
4. Girouard G, Lachance C, Pelletier R. Observations on (1-3)-[beta]-D-glucan detection as a diagnostic tool in endemic mycosis caused by *Histoplasma* or *Blastomyces*. *J Med Microbiol* 2007 Jul; 56(Pt 7):1001-2.
5. Myint T, Chow FC, Bloch KC, et al. Detection of (1,3)-beta-d-Glucan in Cerebrospinal Fluid in *Histoplasma* Meningitis. *J Clin Microbiol* 2018 Oct; 56(10).
6. Thompson GR, III, Bays DJ, Johnson SM, Cohen SH, Pappagianis D, Finkelman MA. Serum (1->3)-beta-D-glucan measurement in coccidioidomycosis. *J Clin Microbiol* 2012 Sep; 50(9):3060-2.
7. Pickering JW, Sant HW, Bowles CAP, Roberts WL, Woods GL. Evaluation of a (1->3)-[beta]-D-Glucan Assay for Diagnosis of Invasive Fungal Infections. *J Clin Microbiol* 2005 Dec 1; 43(12):5957-62.
8. Lyons JL, Thakur KT, Lee R, et al. Utility of Measuring (1,3)-beta-d-Glucan in Cerebrospinal Fluid for Diagnosis of Fungal Central Nervous System Infection. *J Clin Microbiol* 2015 Jan; 53(1):319-22.
9. Mikulska M, Furfaro E, Del B, V, et al. (1-3)-beta-D-Glucan in Cerebrospinal Fluid Is Useful for the Diagnosis of Central Nervous System Fungal Infections. *Clin Infect Dis* 2013 May; 56(10):1511-2.
10. Zangeneh TT, Malo J, Luraschi-Monjagatta C, et al. Positive (1-3) B-d-glucan and cross reactivity of fungal assays in coccidioidomycosis. *Med Mycol* 2015 Feb 1; 53(2):171-3.
11. Stevens DA, Zhang Y, Finkelman MA, Pappagianis D, Clemons KV, Martinez M. Cerebrospinal Fluid (1,3)-Beta-d-Glucan Testing Is Useful in Diagnosis of Coccidioidal Meningitis. *J Clin Microbiol* 2016 Nov; 54(11):2707-10.
12. Shupp JW, Petraitiene R, Jaskille AD, et al. Early serum (1->3)-beta-D-glucan levels in patients with burn injury. *M* 2012 May; 55(3):224-7.
13. Goudjil S, Chazal C, Moreau F, Leke A, Kongolo G, Chouaki T. Blood product transfusions are associated with an increase in serum (1-3)-beta-d-glucan in infants during the initial hospitalization in neonatal intensive care unit (NICU). *J Matern Fetal Neonatal Med* 2017 Apr; 30(8):933-7.
14. Moro H, Koshio N, Bamba Y, et al. The Effect of Intravenous Gamma-globulin Reagents on the Measurement Results of (1,3)-beta-D-glucan. *Kansenshogaku Zasshi* 2017 Jan; 91(1):1-6.