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MYCOLOGY

Detection of (1,3)-β-D-Glucan in Cerebrospinal Fluid in *Histoplasma* Meningitis

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ABSTRACT The diagnosis of central nervous system (CNS) histoplasmosis is often difficult. Although cerebrospinal fluid (CSF) (1,3)-β-D-glucan (BDG) is available as a biological marker for the diagnosis of fungal meningitis, there are limited data on its use for the diagnosis of *Histoplasma* meningitis. We evaluated CSF BDG detection, using the Fungitell assay, in patients with CNS histoplasmosis and controls. A total of 47 cases and 153 controls were identified. The control group included 13 patients with a CNS fungal infection other than histoplasmosis. Forty-nine percent of patients with CNS histoplasmosis and 43.8% of controls were immunocompromised. The median CSF BDG level was 85 pg/ml for cases, compared to <31 pg/ml for all controls ($P < 0.05$) and 82 pg/ml for controls with other causes of fungal meningitis ($P = 0.27$). The sensitivity for detection of BDG in CSF was 53.2%, whereas the specificity was 86.9% versus all controls and 46% versus other CNS fungal infections. CSF BDG levels of ≥80 pg/ml are neither sensitive nor specific to support a diagnosis of *Histoplasma* meningitis.

KEYWORDS (1,3)-β-D-glucan, cerebrospinal fluid, *Histoplasma*, meningitis

The diagnosis of central nervous system (CNS) histoplasmosis is challenging. In one large case series study (1), cerebrospinal fluid (CSF) cultures were positive for only 19.1% of patients, and culture results were often significantly delayed after clinical presentation. More rapid diagnosis could be achieved through detection of antibody (sensitivity, 82.2%) and antigen (sensitivity, 78.0%) in the CSF, with at least one of the tests being positive for 98.0% of patients with CNS histoplasmosis (1). Although a serum assay to detect (1,3)-β-D-glucan (BDG), a fungal cell wall polysaccharide, has been cleared by the U.S. Food and Drug Administration for serological diagnosis of invasive fungal diseases since 2004, the assay is not approved for CSF testing. Elevated levels of BDG have been detected in the CSF of patients with fungal meningitis caused by

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Candida sp. (2, 3), *Aspergillus* (2, 4), *Exserohilum* (5, 6), *Cryptococcus* (4, 7), and *Coccidioides* (8). Data on the utility of BDG in the diagnosis of *Histoplasma* meningitis are limited (4, 9). We evaluated CSF BDG detection using the Fungitell assay in the largest series of patients with CNS histoplasmosis to date.

MATERIALS AND METHODS

Cases were classified using previously defined criteria (1). Patients were categorized as CNS histoplasmosis cases if they had clinical symptoms of meningitis and/or brain imaging abnormalities and supporting laboratory findings, as follows: confirmed CNS histoplasmosis, isolation of *Histoplasma capsulatum* from CSF; probable CNS histoplasmosis, detection of *Histoplasma* antigen by enzyme immunoassay (EIA) or anti-*Histoplasma* antibodies in the CSF by immunodiffusion (ID) or complement fixation (CF); possible CNS histoplasmosis, pulmonary or disseminated histoplasmosis with CSF pleocytosis but without laboratory confirmation of CNS involvement (negative or absent culture findings, microscopy findings, and detection of antigen or antibody by ID or CF in the CSF) and no alternative etiology for the CSF pleocytosis. Controls included patients with pulmonary or disseminated histoplasmosis without CNS involvement (no clinical findings for meningitis, no pleocytosis or CNS imaging abnormalities, and no diagnosis of or treatment for CNS histoplasmosis) or with negative testing for histoplasmosis (either with or without CSF pleocytosis), including patients with meningitis due to fungal pathogens other than *Histoplasma*, nonfungal meningitis, and noninfectious CNS disorders.

BDG levels were measured in the remaining stored CSF specimens using the Fungitell assay, according to the methods used for serum specimens, as reported previously for CSF (4, 5). Data regarding prior treatment were not available when CSF specimens were obtained. According to the manufacturer's guidelines for serum BDG assays, a positive CSF BDG result was defined as ≥80 pg/ml. Chi-square analysis, Student's *t* test, and the step-down Bonferroni multiple-comparison procedure were used to compare subgroups, using MedCalc software.

RESULTS

Forty-seven subjects with CNS histoplasmosis were enrolled in the study, including 9 (19.1%) confirmed, 33 (70.2%) probable, and 5 (10.6%) possible cases. A total of 153 subjects without CNS histoplasmosis were included as controls, including 13 (8.5%) with other causes of fungal meningitis, 31 (20.3%) with nonfungal meningitis, and 109 (71.2%) with noninfectious CNS disorders (e.g., encephalopathy or seizure disorder). Ten of 11 controls with pulmonary or disseminated histoplasmosis had a noninfectious CNS disorder, and 1 had *Toxoplasma* encephalitis. Cultures were positive for fungal pathogens for 6 (5.1%) of 117 controls for whom cultures were performed; pathogens included *Cryptococcus* ($n = 4$), *Aspergillus* ($n = 1$), and *Candida dubliniensis* ($n = 1$). The other 7 fungal meningitis control cases had the following: blastomycosis ($n = 3$) (2 controls were diagnosed by CSF antigen, one with the organism being isolated from bronchoalveolar lavage fluid and the other with characteristic large, broad-based budding yeast consistent with *Blastomyces* being identified with Grocott's methenamine silver staining of leptomeninges from a postmortem specimen; the third control had a positive urine antigen test result and a positive culture for *Blastomyces* from bronchoalveolar lavage fluid), cryptococcosis ($n = 1$, diagnosed by antigen testing), coccidioidomycosis ($n = 1$, diagnosed by antibody testing), aspergillosis ($n = 1$, diagnosed by antigen testing), and candidiasis ($n = 1$, diagnosed by blood culture). Forty-nine percent of patients with CNS histoplasmosis and 43.8% of controls were immunocompromised. BDG levels in the CSF among the different groups are shown in Fig. 1.

CSF BDG levels were not significantly different among the confirmed, probable, and possible cases of *Histoplasma* meningitis ($P = 0.93$) (Table 1). The median BDG level for cases was 85 pg/ml, compared to <31 pg/ml for all controls ($P < 0.05$), <31 pg/ml for nonfungal meningitis ($P < 0.05$), and <31 pg/ml for noninfectious CNS disorders ($P < 0.05$). There were no significant differences in median BDG levels between cases of *Histoplasma* meningitis (85 pg/ml) and other fungal meningitis (82 pg/ml) ($P = 0.27$).

Twenty-five of the 47 *Histoplasma* meningitis cases had CSF BDG levels of ≥80 pg/ml, resulting in a sensitivity of 53.2%. Of the 153 controls, 133 had CSF BDG levels of <80 pg/ml, resulting in an overall specificity of 86.9% for detection of BDG in CSF for CNS histoplasmosis. Using the 140 controls without fungal meningitis, the specificity was 90.7%; using the 11 controls with disseminated or pulmonary histoplasmosis without CNS involvement, the specificity was 100%. Using the controls with other

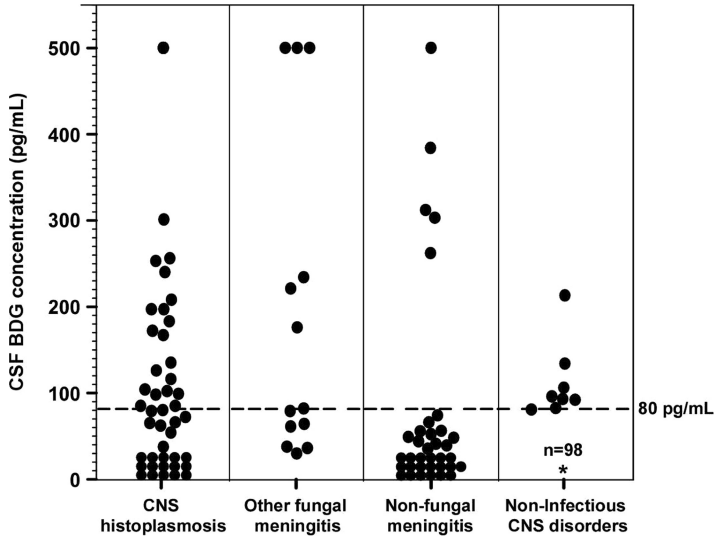

FIG 1 CSF BDG levels in CNS *Histoplasma* meningitis, other fungal CNS infections, nonfungal CNS infections, and noninfectious CNS disorders. The dashed line represents the value of 80 pg/ml. *, There were 98 controls with noninfectious CNS disorders who had CSF BDG levels of <80 pg/ml.

causes of fungal meningitis, however, the specificity was 46.2%. The median CSF BDG level in 6 controls with culture-positive fungal CNS infections other than histoplasmosis was 227 pg/ml, and 5 had levels of ≥80 pg/ml. Among the 7 controls with nonhistoplasmosis fungal CNS infections diagnosed by antigen testing, antibody testing, or blood culture, the median BDG level was 61 pg/ml; 2 had CSF BDG levels of ≥80 pg/ml.

Histoplasma meningitis cases with CSF BDG levels of ≥80 pg/ml were older than those with BDG levels of <80 pg/ml (mean age, 47 years ± 7 versus 36 years ± 8; $P = 0.03$). There were no statistically significant differences in sex ($P = 0.12$), immunocompromised status ($P = 0.89$), positive CSF culture ($P = 0.56$), positive *Histoplasma* antigen testing ($P = 0.51$), or the presence of high *Histoplasma* antigen levels (>19 ng/ml) ($P = 0.22$) between cases with BDG levels of ≥80 and those with levels of <80 pg/ml.

Among 37 patients with positive CSF *Histoplasma* antigen testing, the median BDG level was 127.6 pg/ml; 21/37 patients (56.8%) had levels of ≥80 pg/ml, and 25/37 patients (67.6%) had levels of >31 pg/ml. Four of 25 cases with BDG levels of ≥80 pg/ml and 7 of 32 cases with BDG levels of >31 pg/ml had negative CSF *Histoplasma* antigen testing. Two cases had positive CSF antigen test results that were below the detectable limit of <0.4 ng/ml.

CSF BDG levels of ≥80 pg/ml were also detected in 13 patients with bacterial meningitis or a brain abscess ($n = 3$), viral encephalitis ($n = 1$), Rocky Mountain spotted fever ($n = 1$), stroke ($n = 2$), neurosarcoidosis ($n = 1$), melanoma ($n = 1$), hypoxic brain

TABLE 1 CSF BDG levels in different groups of *Histoplasma* meningitis cases and controls

Group	No. of subjects	CSF BDG level (median [IQR]) (pg/ml)	No. (%) with BDG level of ≥80 pg/ml	*P*
All cases	47	85 (31–194)	25 (53.2)	Reference
Confirmed	9	116 (62–197)	5 (55.6)	0.93[a]
Probable	33	85 (31–183)	18 (54.5)	
Possible	5	72 (54–99)	2 (40.0)	
All controls	153	<31 (<31 to 55)	20 (13.1)	<0.05[b]
Other fungal meningitis	13	82 (61–234)	7 (53.8)	0.27[b]
Nonfungal meningitis	31	<31 (<31 to 55.5)	5 (16.1)	<0.05[b]
Noninfectious CNS disorder	109	<31 (<31 to 44)	7 (6.4)	0.05[b]

[a]*P* for comparison of confirmed, probable, and possible cases.
[b]*P* for comparison with cases.

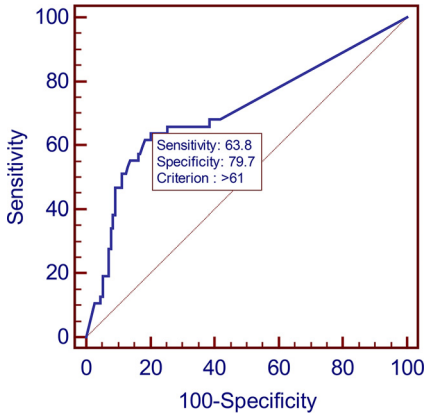

FIG 2 ROC curve of CSF BDG levels to distinguish histoplasmosis cases from all controls, including other fungal meningitis controls. The AUC was 0.706.

injury ($n = 1$), acute psychosis ($n = 1$), a seizure disorder ($n = 1$), or an adverse reaction to medication ($n = 1$). The median BDG level was 134 pg/ml (interquartile range [IQR], 93 to 303 pg/ml).

Using the Youden method (10) for receiver operating characteristic (ROC) analysis, the optimal cutoff value for CSF BDG levels was 61 pg/ml for CNS histoplasmosis versus controls, including other fungal meningitis cases, with sensitivity of 63.8%, specificity of 79.7%, and area under the curve (AUC) of 0.706 (Fig. 2). When other fungal meningitis cases were excluded, the optimal cutoff value for BDG was 58 pg/ml, yielding sensitivity and specificity of 67.8% and 83.7%, respectively, for *Histoplasma* meningitis, compared with nonfungal meningitis controls, and AUC of 0.767 (Fig. 3).

DISCUSSION

This is the first large case series study to evaluate the detection of BDG in the CSF of patients with *Histoplasma* meningitis. In this study, using the manufacturer's recommended cutoff value of ≥80 pg/ml, the sensitivity was 53.2% and the specificity was 86.9% when all controls were used. However, the specificity fell to 46% when only controls with other types of fungal meningitis were used.

The optimal cutoff value is not well defined for CSF BDG levels. For serum BDG levels, the assay manufacturer recommends that <60 pg/ml be interpreted as negative, 60 pg/ml to 79 pg/ml as intermediate, and ≥80 pg/ml as positive (11). Some authors (6, 7) used ≥80 pg/ml as a cutoff value for CSF BDG levels, whereas one author (8) used >31 pg/ml.

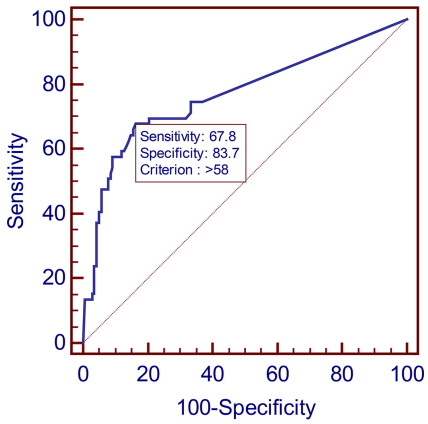

FIG 3 ROC curve of CSF BDG levels to distinguish histoplasmosis cases from controls except for other fungal meningitis controls. The AUC was 0.767.

The sensitivity was lower than that for other types of fungal meningitis, such as *Exserohilum* meningitis (84%) (5), cryptococcal meningitis (89%) (7), and coccidioidal meningitis (96%) (8). The overall specificity in this study (87%) was comparable to that reported for *Exserohilum* meningitis (95%) (5), cryptococcal meningitis (85%), and coccidioidal meningitis (85%) (8), although the specificity of a diagnostic test depends on the controls selected for comparison. BDG is not specific for *Histoplasma*, as evidenced by the low specificity in comparison with other fungal meningitis controls. However, CSF BDG levels may help distinguish a fungal CNS process from a nonfungal process, based on the specificity of 90.7% when the cases were compared with controls with a nonfungal neurological diagnosis. In addition, CSF BDG levels below the cutoff value can help reassure clinicians that a patient with disseminated or pulmonary histoplasmosis does not have CNS involvement.

We found that the sensitivity of detection of CSF BDG in *Histoplasma* meningitis was lower than that of detection of BDG in other forms of fungal meningitis. *Histoplasma* yeasts secrete β-1,3-glucanases that remove exposed cell wall β-glucans to minimize host detection of *Histoplasma* yeasts (12), which may explain the lower sensitivity of CSF BDG testing in *Histoplasma* meningitis, compared to the other causes of fungal meningitis. Lower fungal burdens in the CSF in CNS histoplasmosis also might lead to lower sensitivity of CSF BDG testing.

CSF BDG was also detected in 13 patients without a fungal CNS infection. The reason for high CSF BDG levels in these nonfungal meningitis controls is unclear but could represent false-positive results due to cross contamination at the time of processing and testing, surgical gauze in the lumbar puncture kit (13), or the use of certain antibiotics (14). False-positive serum BDG results can also occur with a history of hemodialysis, blood transfusion, or intravenous immunoglobulin therapy.

Limitations of the study include its retrospective design and limited clinical and laboratory data. For some subjects, CSF fungal culture, antigen detection, and antibody detection were not performed as part of clinical care, and thus results were not able to be analyzed. Paired serum BDG testing results were not available. In summary, CSF BDG levels of ≥80 pg/ml are not specific for a diagnosis of *Histoplasma* meningitis. Furthermore, CSF BDG levels of <80 pg/ml cannot reliably rule out a diagnosis of CNS histoplasmosis.

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