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Utility of Measuring (1,3)-β-D-Glucan in Cerebrospinal Fluid for Diagnosis of Fungal Central Nervous System Infection

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(1-3)-β-D-Glucan (BDG) from cerebrospinal fluid (CSF) is a promising marker for diagnostic and prognostic aid of central nervous system (CNS) fungal infection, but its relationship to serum values has not been studied. Herein, we detected BDG from CSF at levels 2-fold lower than those in serum in patients without evidence of fungal disease but 25-fold higher than those in in serum in noncryptococcal CNS fungal infections. CSF BDG may be a useful biomarker in the evaluation of fungal CNS disease.

BDG [(1,3)-β-D-glucan] is a fungal cell wall polysaccharide (*Cryptococcus* and mucoralean fungi generally excepted). Circulating BDG is sometimes detectable at low levels (<60 pg/ml) in blood of asymptomatic individuals and is elevated in patients with invasive fungal infections (1). The U.S. Food and Drug Administration (FDA) has approved testing of BDG in serum to aid in diagnosis of invasive fungal infections. Clinical studies have demonstrated that the test marketed in the United States has a sensitivity of 75 to 77% and a specificity of 60 to 80% in the diagnosis of candidiasis in intensive care unit patients and aspergillosis in lung transplant patients with a cutoff value of ≤60 pg/ml as negative and ≥80 pg/ml as positive (2–4).

Cerebrospinal fluid (CSF) BDG measurement has not been validated. Its utility was first described for a nonneutropenic rabbit model of experimental hematogenous *Candida* meningoencephalitis (5). Lyons et al. (6) and Litvintseva et al. (7) showed its diagnostic and prognostic utility during the 2012 U.S. fungal central nervous system (CNS) infection outbreak. Our study further explores the utility of CSF BDG and the relationship between serum and CSF BDG in CNS fungal and nonfungal disease.

We selected CSF and serum samples collected for routine care between 2007 and 2013 and frozen as part of a research protocol approved by Johns Hopkins Institutional Review Board. We selected subjects whose CSF and serum were collected within 24 h of one another. There was only one serum and one CSF specimen available per patient. Diagnoses were confirmed by chart review and were simultaneous with the timing of sample acquisition.

Demographic information, including gender, age, race, clinical diagnosis, and indication for lumbar puncture, was documented. Pertinent clinical information gathered included history of fungal infection or other CNS infection, HIV status, and history of organ transplantation or other evidence of immunosuppression. Culture, serology, histopathology, and neuroimaging data were collected for diagnostic purposes. Fungal infections were defined as proven, probable, or possible based on EORTC/MSG criteria (8) or CDC definitions for outbreak-associated meningitis (9).

Sterilely collected CSF and serum specimens had been frozen to −80°C and were shipped on dry ice to the Beacon Diagnostics Laboratory for testing via the Fungitell assay (Associates of Cape Cod, Inc., East Falmouth, MA). All assays were performed in a biosafety cabinet that had not been used to manipulate fungal cultures. Glucan-free dilution tubes and pipette tips were used. CSF and serum samples were equilibrated to room temperature, vortexed, and tested in duplicate or triplicate using the kit manufacturer protocol. The final result was the mean of the replicate or triplicate readings. Spectrophotometric analysis was performed using a BioTek ELx808IU microplate reader (BioTek Instruments Inc., Winooski, VT). Results were compared to a standard curve derived from serially diluted standard provided with the limit of detection to <31 pg/ml. Samples with titers above 500 pg/ml were diluted and retested. Samples with titers below 31 pg/ml were rerun with extended low-range standard curves (3.9 pg/ml to 125 pg/ml). Individuals performing the assay were blinded to all diagnostic and clinical information.

Patient demographic and clinical characteristics were summarized using appropriate descriptive statistics, e.g., counts and percentages, median and interquartile range (IQR). Nonparametric analysis methods were used due to a non-Gaussian distribution of values. Patients with no evidence of CNS fungal infection were compared to those with probable and proven CNS fungal disease using Wilcoxon rank-sum tests. Wilcoxon signed-rank tests were used to determine the statistical differences between levels of BDG in paired CSF and serum. Spearman correlation coefficients were used to determine the relationship between BDG levels and CSF pleocytosis. Logistic regression analysis and receiver operating

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TABLE 1 Levels of BDG in paired serum and CSF samples of patients with or without CNS fungal infection

Disease category	No. of patients or case no.	BDG in paired serum and CSF samples				No. of patients with:	
		Level in serum (pg/ml)[a]	Level in CSF (pg/ml)[a]	*P* value[b]	Serum/CSF BDG ratio[a]	Pleocytosis	HIV
Nonfungal infection							
Multiple sclerosis	9	20 (10–35)	9 (4–28)	0.02	2.22 (0.77–6.2)	6	0
Primary headaches	10	27.5 (12–64)	15.5 (<4–27)	<0.02	2.85 (0.86–5.63)	0	4
Pseudotumor cerebri	5	28 (18–58)	17 (13–43)	0.31	1.54 (0.65–2.9)	0	0
Other CNS abnormalities	24	28 (11–1334)	14 (<4–109)	0.10	2.36 (0.30–45.3)	0	4
Bacterial/viral CNS infection	18	25.5 (7–397)	13 (<4–71)	0.07	2.38 (0.31–14.3)	18	10
Subtotal	66	26.5 (7–1344)	13.5 (<4–109)	<0.001	2.24 (0.30–45.3)		
Non-CNS fungal infections							
Proven invasive candidiasis	No. 1	1,051	27		38.9	0	1
Probable invasive aspergillosis	No. 2	894	40		22.4	0	1
Proven fungal sinusitis/mastoiditis[d]	No. 3	201	57		3.5	0	0
Possible fungal sinusitis[e]	No. 4	11	685		0.02	0	0
Probable disseminated coccidioidomycosis	No. 5	14	139		0.10	0	1
Probable *Pneumocystis* pneumonia	No. 6	1179	158		7.5	1	1
Probable fungal pneumonia	No. 7	2,100	34		61.8	0	1
Probable invasive candidiasis	No. 8	260	64		4.1	0	0
Probable invasive candidiasis	No. 9	229	5		45.8	0	0
Probable invasive candidiasis	No. 10	685	7		97.9	0	0
Subtotal	10	472.5 (11–2,100)	48.5 (5–685)	0.03	14.9 (0.02–97.9)		
CNS fungal infections							
Cryptococcus meningitis	No. 1 (<1:5[c])	30	<4			1	1
	No. 2 (1:160[c])	16	37		0.43	1	1
	No. 3 (<1:5[c])	27	21		1.29	1	0
	No. 4 (1:80[c])	91	53		1.72	1	0
	No. 5 (1:40[c])	34	32		1.06	1	0
	No. 6 (1:10[c])	15	33		0.45	1	1
Probable cerebral histoplasmosis[f]	No. 7	14	110		0.13	1	1
Proven *Exserohilum* meningitis (outbreak patient)	No. 8	32	797		0.04	0	0
Probable *Exserohilum* meningitis (outbreak patient)	No. 9	33	1,524		0.02	1	0
Total	85					33	27

[a] Median (range) for group data or value for individual data.
[b] *P* value was determined by Wilcoxon signed-rank test.
[c] CSF cryptococcal antigen titer determined by a lateral flow assay (IMMY, Oklahoma, USA). All six patients (case no. 1 to 6) had positive serum cryptococcal antigen results by the lateral flow assay.
[d] This fungal sinusitis case was proven based on histology examination. The right mastoid excision tissue from the patient showed fungal hypha forms. However, culture was negative.
[e] This patient had chronic sinusitis refractory to multiple courses of antibiotics empirically and was diagnosed clinically as having possible fungal sinusitis.
[f] This patient tested positive for *Histoplasma* antigen in CSF.

characteristic (ROC) curves were plotted to determine the best cutoff value for CSF BDG to diagnose CNS fungal infection. Sensitivity, specificity, and exact binomial 95% confidence intervals (CIs) for each were calculated. Analyses were performed using the software program SAS version 9.3 (SAS Institute, Inc., Cary, NC). All tests were two sided and considered significant at a *P* value of <0.05.

Paired serum and CSF samples were available for 92 patients. The median (range) age was 47 (18 to 97) years; 44 (52%) were female, 39 (46%) African-American, and 39 (46%) Caucasian. Twenty-seven (32%) patients were HIV infected, with median (range) CD4 count of 98 (1 to 320) cells/μl and HIV plasma RNA of 102,000 (<20 to 406,000) copies/ml.

Clinical characteristics of the patients are presented in Table 1. A total of 66 patients were categorized as having nonfungal infection, 10 had proven, probable, or possible fungal infection with no evidence of CNS involvement, 3 had proven or probable noncryptococcal CNS fungal infection, and 6 had definitive cryptococcal meningitis (CM). Seven patients were excluded from analysis because their BDG testing results were inconclusive due to the presence of interfering substances.

Ten of 66 (15%) without fungal infection had undetectable CSF BDG levels (<4 pg/ml), and 56 had detectable levels, ranging from 4 to 109 pg/ml. Median CSF BDG was lower than that of paired serum ($P < 0.001$), with a serum-CSF BDG ratio of 2.24 (range, 0.3 to 45.3) (Table 1).

All 10 non-CNS proven, probable, or possible fungal infection patients had measurable CSF BDG, but the median was lower than that in serum ($P = 0.03$), with a median ratio of BDG in serum to that in CSF of 14.9 (range, 0.02 to 97.9) (Table 1).

In the six patients with CM infection, 5 (83%) had measurable CSF BDG levels that were comparable to serum values. The median ratio of serum BDG to CSF BDG was 1.17 (range, 0.43 to 7.5).

In the three patients with proven and probable CNS fungal infection, all had detectable CSF BDG, ranging from 110 to 1,524 pg/ml, levels which were much higher than those of serum BDG (serum BDG to SF BDG, 0.04 [0.02 to 0.13]).

ROC analysis for CSF BDG values demonstrated a cutoff point of 110 pg/ml. At this value, sensitivity was 100% (95% CI = 29 to 100%), and specificity was 96% (95% CI = 89 to 98%), with an area under the curve of 0.982.

Patients with probable or definitive CNS fungal infection had significantly higher CSF BDG levels ($P = 0.01$) and lower serum-CSF BDG ratios ($P = 0.01$) than patients with nonfungal CNS disease. Serum BDG was not significantly different between the groups ($P = 0.94$).

There were no CSF BDG differences when analyzed by presence ($n = 33$) or absence ($n = 52$) of CSF pleocytosis ($P = 0.68$). Among HIV patients ($n = 27$), there was no difference between those with ($n = 14$) and without ($n = 13$) CSF pleocytosis ($P = 0.62$).

We detected low CSF BDG levels in patients without evidence of fungal infection. CSF BDG levels remained low in non-CNS fungal infection but were significantly higher in three cases of CNS fungal infection. The CSF BDG level was lower than that in paired serum for those without evidence of fungal infection, implying that the positive cutoff value for CSF may be lower than that for serum. However, the level of BDG was significantly higher in serum than in CSF in patients with non-CNS proven/probable/possible fungal infection versus results for those with CNS fungal infections. This suggests the integrity of the blood-brain barrier in walling off CNS fungal infections, preventing spillover. Conversely, we found that the BDG level was significantly higher in CSF than in serum in three cases of proven/probable CNS fungal infection. This could suggest isolated CNS infection without significant spillover into venous blood or that BDG may be more rapidly cleared in blood than in CSF, as indicated in the fungal meningitis rabbit model (5), and it suggests that serum values may not be helpful in determining invasion of the CNS by a fungal pathogen.

It is not clear why BDG levels were higher in CSF than in serum in case numbers 4 and 5 with non-CNS fungal infections (Table 1). In case number 4, with possible fungal sinusitis, by sheer proximity of the sinuses to the dura and arachnoid and due to the chronicity of infections in the patient, it is plausible that the process had extended to the subarachnoid space. This notion has been documented in bacterial meningitis resulting from extension of sinusitis and in rhinocerebral mucormycosis, which frequently arises from extension of a sinus infection. In case number 5, with probable coccidioidomycosis, possible involvement of CNS cannot be excluded.

Similar to that found by Litvintseva (7), our cutoff of 110 pg/ml demonstrates both high sensitivity and specificity of CSF BDG, although given the low number of true positives, the former is difficult to interpret. Our study adds comparison of CSF BDG to serum BDG, and our findings suggest that testing of CSF may be more useful than that of serum in evaluating CNS disease.

In the *Exserohilum* outbreak cases, CSF BDG levels anecdotally did not correlate with CSF pleocytosis (6, 7). We found the same here. Likewise, in HIV patients with pleocytosis, CSF BDG was not elevated, suggesting that the degree of inflammation and fungal BDG shedding may be disparate.

Despite the fact that *Cryptococcus* produces little to no BDG, studies have reported detectable serum BDG in cryptococcosis (4, 10, 11). CSF BDG in CM had not been assessed until a recent, proven case with CSF BDG of 331 pg/ml and paired serum BDG of <7 pg/ml (12). In our six CM patients (with cryptococcal antigen titers ranging from $<1{:}5$ to 1:160; three patients were HIV positive [Table 1]), BDG was detectable but at low and comparable levels in serum and CSF, suggesting limited utility in this disease, although additional factors, such as the fungal disease burden, may account for this discrepancy. In fact, rapid and accurate diagnosis of the disease can be reliably achieved by detection of cryptococcal antigen in CSF.

There are several limitations to this study, including its retrospective design; therefore, circumstances that might give rise to false-positive results were not controlled for, especially since the role of the blood-brain barrier in preventing BDG transport across it has not been delineated. These circumstances include but are not limited to a history of hemodialysis, blood transfusion, intravenous immunoglobulin use, and use of certain antibiotics. Additionally, although we found no differences in BDG levels from serum versus CSF between those with and without HIV, it is difficult to know the effect HIV may have. Finally, the small number of CNS fungal infection cases precludes generalizability of our findings and can be used only to call for further study.

Our findings suggest that elevated CSF BDG could be useful for diagnosing or excluding fungal CNS infections. Additionally, it may be an important determinant of fungal disease well before organism growth in culture. Testing of larger cohorts is necessary to determine whether CSF BDG can be used to aid in diagnosis, and predictive capabilities as well as evaluation for false positives need to be determined.

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