Cerebrospinal fluid (1,3) β-D-glucan detection as an aid to diagnose iatrogenic fungal meningitis

(Running title: cerebrospinal fluid β-D-glucan in fungal meningitis)

Jennifer L. Lyons MD1*, Karen L. Roos MD6*, Kieran A. Marr MD2,3, Henry Neumann MD2, Julie B. Trivedi MD2, Dorlan J. Kimbrough MD1, Lisa Steiner PA-C4, Kiran T. Thakur MD1, Daniel M. Harrison MD1, and Sean X. Zhang MD, PhD5#

Johns Hopkins University School of Medicine, Department of ¹Neurology, ²Medicine, ³Oncology, ⁴Emergency Medicine, and ⁵Pathology, Baltimore, MD and ⁶Indiana University School of Medicine, Department of Neurology, Indianapolis, IN

* Authors contributed equally to this work

#Corresponding author

Dr. Sean X. Zhang
Division of Medical Microbiology
Department of Pathology
The Johns Hopkins University School of Medicine
The Johns Hopkins Hospital
600 North Wolfe Street, Meyer B1-193
Baltimore, MD 21205
Email: szhang28@jhmi.edu
Tel: 410-955-5077
Fax: 410-614-8087
Abstract

This case series highlights experience with use of the Fungitell® assay for quantifying (1,3) β-D-glucan on cerebrospinal fluid during the current U. S. outbreak of fungal meningitis related to contaminated methylprednisolone acetate. This test may prove a useful adjunct in diagnosis and management of exposed patients.
β-D-glucan (BG) is found in cell walls of multiple fungi. Its detection in serum assists in diagnosis of invasive fungal infections (1). Recently, diagnostic challenge has arisen in the fungal meningitis outbreak associated with exposure to contaminated epidural steroid injections (2). Diagnosis in this setting has been established by culture of cerebrospinal fluid (CSF) and/or detection using a pan-fungal polymerase chain reaction (PCR) assay performed by the Centers for Disease Control (CDC). However, these tests have not always been positive in suspected cases (3). One early study has demonstrated the proof-of-concept of using CSF BG detection in diagnosis of fungal central nervous system infection in an experimental hematogenous Candida meningoencephalitis model (4). Herein we report our experience with CSF BG measurement in 5 individuals from Johns Hopkins Hospital and Indiana University Hospital who were exposed to potentially contaminated drugs. Cases were diagnosed and managed according to CDC guidelines. BG was tested at Beacon Diagnostics Laboratory (East Falmouth, MA) using Fungitell® assay. Information was obtained by chart review with approval from Johns Hopkins Institutional Review Board.

The first case is a 55-year-old woman who developed headaches, blurred vision, and injection site pain one week after lumbar epidural injection with potentially contaminated methylprednisolone and was admitted 35 days after symptom onset when the outbreak was recognized. CSF showed 30 WBCs/mm³ and normal glucose and protein; no opening pressure was recorded. Intravenous voriconazole was initiated but symptoms continued despite troughs 2 – 3 mcg/mL. Repeat lumbar puncture (LP) showed opening pressure of 42 cm H₂O, 974 WBC/mm³ (56% neutrophils, 16%
lymphocytes, 21% monocytes), 1000 red blood cells (RBCs)/mm$^3$, normal glucose, and protein 93 mg/dL, with a negative culture. CSF PCR performed by the CDC was negative. With serial LPs for persistent headache and elevated opening pressures and voriconazole increase to maintain troughs 3-5 mcg/mL, her symptoms resolved. CSF fungal cultures from post-injection days 57, 69, and 73 were negative. CSF BG sent on post-injection days 57 and 73 were positive at 2,396 and 701 pg/mL, respectively (Table 1).

The second case is a 37-year-old man who underwent lumbar epidural injection with potentially contaminated methylprednisolone and developed headache several days later. Thirteen days after the injection he started oral voriconazole. His symptoms did not improve, and LP 2 weeks later showed 5 WBCs (Table 1). PCR performed by the CDC and fungal cultures were negative. MRI of the brain showed a small intraparenchymal hemorrhage in the left frontal lobe; there was no abnormal enhancement, and MRA of the head was normal. Given the lesion’s imaging characteristics, a family history of cerebral vascular malformations, and close follow up, the cause was thought to be cavernous malformation bleeding, and voriconazole was stopped. CSF BG was below the detection limit (<31 pg/mL). His headaches resolved and did not return after voriconazole cessation.

The third case is a 66-year-old woman who developed headache, fever, and injection site pain 19 days after lumbar injection from implicated methylprednisolone lot. Lumbosacral MRI was unremarkable. On post exposure day 38, CSF showed 54% neutrophilic pleocytosis with 32 WBC/μL, elevated protein at 90 mg/dL and normal.
glucose; she was admitted and voriconazole initiated. Fungal culture and PCR performed at CDC were negative. Repeat MRI showed cauda equina enhancement and injection site edema. She was discharged after 2 weeks but self-discontinued voriconazole due to nausea and diarrhea; symptoms returned 2 weeks later. BG was detectable in CSF from day 72 at 96 pg/mL (Table 1). Voriconazole was re-initiated and symptoms resolved with troughs 6 mcg/mL.

The fourth case is a 45-year-old woman with a history of epidural injection from an implicated methylprednisolone lot who developed rhinorrhea, cough, and sore throat more than 6 weeks after her injection, progressing over several days to include headache and mild neck stiffness. Her symptoms were thought to be due to a respiratory virus. Fungal culture was negative, and fungal PCR was not performed given lack of pleocytosis (Table 1). CSF BG was below the detection limit.

The fifth case is a 65-year-old man who received lumbar epidural steroid injections on 5/7/12, 7/16/12, and 9/4/12 with methylprednisolone from contaminated lots. He was apprised in mid-October and requested evaluation prior to a previously scheduled surgery. His neurologic examination was normal, and he was asymptomatic. Serum and CSF galactomannan (Platelia™, Bio-Rad Laboratories) were negative (index <0.5). The BG was positive in serum at 183 pg/mL and detectable in CSF at 39 pg/mL (Table 1). Intravenous voriconazole therapy was initiated. A lumbar MRI showed a discitis at L1-L2 and 3 mm abnormal enhancement in the ventral epidural space that was new since 10/5/12. After 2 months of voriconazole, the fluid collection resolved, and serum BG decreased to 88 pg/mL.
Confirmed cases of fungal meningitis in the current outbreak have primarily involved *Exserohilum rostratum* (3, 5-8). However, most have remained clinically “probable” due to lack of laboratory confirmation. Culture has relatively low sensitivity, confirming only one-third of reported cases (3). The performance of the PCR is not yet known. Cases 1, 3, and 5 had symptoms, CSF pleocytosis, and/or abnormal imaging suggestive of infection but negative fungal cultures and PCR; all three had detectable CSF BG, suggesting fungal involvement. In cases 2 and 4, CSF BG was negative, and alternative diagnoses were considered likely based on clinical presentation, CSF studies, and/or radiographic findings. It is noticeable that CSF BG was detectable in case 5 in the absence of pleocytosis. As fluctuations in CSF WBC count have been noted during this outbreak (unpublished data), it is possible that the Fungitell assay may be very sensitive in detecting fungal elements in the CSF without pleocytosis. In the absence of culture and PCR results, BG detection in CSF raises the possibility of its use as an adjunct to aid in the diagnosis especially while this current epidemic continues to pose diagnostic challenges. Additionally, serum BD may be useful in detection of paraspinal infections that have not penetrated the CSF.

Detection of CSF BG has not been cleared by the FDA as an aid to diagnosis of fungal meningitis, and the appropriate quantitative cut-off for positivity in CSF is unknown (9, 10). It is possible that CSF BG concentration may be lower than that of serum for this assay as illustrated in Case 5 (none of the other cases had serum BG tested during hospitalization), but more data would be necessary to confirm. One study suggested that BG in undetectable in normal CSF (11). False positive test results occur in
serum assays in association with a number of conditions (12-14). We do not yet know whether CSF false positives would occur due to biologic variables in the CSF or from other events in sample processing such as use of cotton gauze or a cotton alcohol swab to clean the puncture site in the LP procedure, but these cases illustrate association between detectable results and suspected disease.

Sequential tests in CSF were available in case 1 and noted to be persistently positive at a high but decreasing values during treatment. This could be potentially useful for determination of therapy duration as measuring CSF BG has been demonstrated as a useful tool for monitoring therapeutic response in an animal model (4), but more data would be necessary to understand the anticipated kinetics of antigen positivity and to provide guidance for treatment duration.

In conclusion, establishing the diagnosis of fungal meningitis in the current nationwide outbreak has been difficult, and little is known about the natural history of this disease or therapeutic responses. Although more studies clearly are necessary to determine normal ranges and to confirm utility for widespread application, CSF BG measurement may be a useful adjunctive to assist diagnosis and management of fungal meningitis cases during this current outbreak.
Acknowledgements. The authors thank Dr. Malcolm Finkelman (Beacon Laboratories) for contribution and helpful discussion and Dr. Stefan Riedel and Richard Lee for assistance with sample processing.

Financial Support. This work was supported by N.I.H. K24 AI085118 (K.A.M).

Conflicts of Interest. K.A.M. reports receiving funds for consultancy / advisory board activities with Astellas, Merck, Pfizer, and UpToDate, and research grants from Astellas, Merck, and Pfizer. S.X.Z received research contract funds from IBIS Biosciences/Abbott Molecular and AdvanDx Corp. J.L.L., K.L.R., H.N., J.B.T., D.J.K., L.S., K.T.T., and D.M.H. report no disclosures.
172 References


Table 1. Demographic and CSF characteristics of cases.

<table>
<thead>
<tr>
<th>Case #</th>
<th>1-a</th>
<th>1-b</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>55</td>
<td>37</td>
<td>66</td>
<td>45</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Symptoms</td>
<td>HA, ISP, blurred vision</td>
<td>HA, ISP</td>
<td>HA, ISP</td>
<td>URI</td>
<td>HA, neck stiffness</td>
<td>None</td>
</tr>
<tr>
<td>Symptom onset*</td>
<td>~7 days</td>
<td>~4 days</td>
<td>19 days</td>
<td>NA</td>
<td>~42 days</td>
<td>NA</td>
</tr>
<tr>
<td>Voriconazole initiation*</td>
<td>45 days</td>
<td>13 days</td>
<td>38 days</td>
<td>NA</td>
<td>50 days</td>
<td></td>
</tr>
<tr>
<td>Day CSF collected*</td>
<td>57 days</td>
<td>73 days</td>
<td>28 days</td>
<td>72 days</td>
<td>~42 days</td>
<td>50 days</td>
</tr>
<tr>
<td>CSF WBC (cells/mm³)</td>
<td>1224</td>
<td>72</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CSF Diff</td>
<td>69% PMN, 13% lymph, 18% mono</td>
<td>43% PMN, 6% lymph, 51% mono</td>
<td>50% lymph, 50% mono</td>
<td>87% lymph, 13% mono</td>
<td>100% lymph</td>
<td>NA</td>
</tr>
<tr>
<td>CSF RBC (cells/mm³)</td>
<td>2793</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>CSF protein (mg/dL)</td>
<td>164</td>
<td>53</td>
<td>48</td>
<td>37</td>
<td>26</td>
<td>49</td>
</tr>
<tr>
<td>CSF glucose (mg/dL)</td>
<td>55</td>
<td>46</td>
<td>74</td>
<td>54</td>
<td>67</td>
<td>52</td>
</tr>
<tr>
<td>CSF fungal culture</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>CSF fungal PCR (CDC)</td>
<td>Neg**</td>
<td>Neg**</td>
<td>Neg</td>
<td>Neg**</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CSF (1,3) β-D-glucan (pg/mL)</td>
<td>2396</td>
<td>701</td>
<td>&lt;31</td>
<td>96</td>
<td>&lt;31</td>
<td>39</td>
</tr>
</tbody>
</table>

HA: headache; ISP: Injection site pain; URI: upper respiratory illness symptoms (rhinorrhea, cough, pharyngitis); PMN: polymorphonuclear cells (neutrophils); lymph: lymphocytes; mono: monocytes; UD: undetectable (<31pg/mL); Neg: negative.

* days after epidural steroid injection date  ** sent from prior CSF sample