Detection of High CSF Levels of (1→3)-Beta-D-glucan in Cryptococcal Meningitis

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Abstract

Background: (1→3)-Beta-D-glucan (BDG) is a helpful diagnostic marker for many invasive fungal infections. However, BDG is not thought to be useful in diagnosing cryptococcosis. We evaluated the utility of BDG as an adjunct diagnostic tool for HIV-infected patients with suspected cryptococcal meningitis.

Methods: BDG concentrations were measured in CSF (n=177) and serum (n=109) of HIV-infected Ugandans and South Africans with suspected meningitis using the Fungitell® assay. Correlations between BDG concentrations and quantitative CSF cryptococcal cultures, CSF cryptococcal antigen (CRAG) titers, and 18 different CSF cytokine concentrations were assessed using non-parametric tests. Mixed models evaluated longitudinal changes in CSF BDG concentrations. Survival analyses evaluated BDG’s relationship with mortality.

Results: The Fungitell® BDG assay provided 89% sensitivity and 85% specificity in CSF for cryptococcal meningitis. Serum sensitivity was suboptimal (79%). CSF BDG concentrations at diagnosis were median (IQR) 343 (200-597) pg/mL in cryptococcal patients and 37 (23-46) pg/mL in patients without cryptococcus. Sensitivity in CSF improved to 96% (53/54) when initial fungal burdens were ≥10,000 colony forming units (CFU)/mL. BDG normalized rapidly following antifungal therapy. Baseline BDG concentrations correlated with CSF fungal burden (rho=0.820, P<0.001) and CSF CRAG lateral flow assay titers (rho=0.780, P<0.001). In cryptococcal meningitis patients, a BDG concentration ≥350 pg/mL was associated with increased 10-week mortality.

Conclusion: BDG is detectable in the CSF of HIV-infected patients with Cryptococcus, and may provide useful prognostic information. Further research is needed to understand the role of BDG in the immunology and management of cryptococcal disease.

Study Population

Figure 1: Analysis Cohort:
- 177 HIV-infected persons enrolled into two prospective cohorts of hospitalized patients with suspected meningitis from 2010-2013.
- 117 specimens obtained at time of diagnosis used in primary analysis.
- 60 specimens obtained used to assess rate of BDG clearance from CSF.
- 67% (78/117) of specimens obtained at diagnosis were from patients with CM.
- Patients diagnosed with CM in Uganda (N=53) or Cape Town, South Africa (N=25)

Methods

CSF was collected by lumbar puncture at diagnosis or 3-20 days after diagnosis and stored at -80°C and shipped on dry ice.

The Fungitell® assay (Associates of Cape Cod, Inc., Falmouth, MA) is used to test CSF for BDG. BDG was detected by Fungitell in CSF of HIV-infected patients.

• BDG levels correlated with baseline levels of both:
  - CRAG fungal burden by quantitative culture (rho=0.820, P<0.001)
  - CRAG LFA titers (rho=0.780, P<0.001)

Figure 2: BDG levels in CSF correlate with fungal burden. (1→3)-Beta-D-glucan concentrations relative to quantitative cryptococcal cultures among all 78 patients diagnosed with cryptococcal meningitis at diagnosis (hollow circle), and day 3 (hollow diamond), day 7 (square), day 10 (hollow triangle) and day 14 (small x) (n=137).

Table 1: Performance of the Fungitell® BDG assay in CSF for the diagnosis of cryptococcal meningitis. The assay provided good sensitivity and specificity when compared to the respective reference standard of culture or CRAG LFA.

Table: Sensitivity of BDG by quantitative CSF fungal culture at time of diagnosis, using a positive cutoff value of 280 pg/mL. Sensitivity improved with higher initial fungal burden by quantitative culture (QCC). Better sensitivity of BDG in CSF was observed in Uganda (mean QCC125,000 CFU/mL) as compared to South Africa (mean QCC 26,000 CFU/mL).

Table: 2: Sensitivity of BDG by quantitative CSF fungal culture at time of diagnosis, using a positive cutoff value of 280 pg/mL. Sensitivity improved with higher initial fungal burden by quantitative culture (QCC). Better sensitivity of BDG in CSF was observed in Uganda (mean QCC125,000 CFU/mL) as compared to South Africa (mean QCC 26,000 CFU/mL).

Conclusions

• BDG can be detected in the CSF of AIDS patients with CM
  - Sensitive and specific for diagnosis of CM in HIV patients.
  - Sensitivity increases with initial fungal burden.
  - BDG rapidly normalizes with effective CM treatment.
  - May be useful in confirming treatment effectiveness, or
  - Identifying relapse cases.

• BDG testing in CSF may provide helpful adjunctive diagnostic information in evaluating HIV patients with meningitis.

• CM should be considered in all immunosuppressed patients with a positive BDG test result using the Fungitell® BDG assay.

• Additional studies are necessary to confirm our observations.

Figure 3: BDG concentrations by meningitis diagnosis and effect of antifungal therapy on BDG concentrations. The median BDG levels in CM patients (gray boxes) was above the assay positive cutoff value of 80 pg/mL (dashed line) at diagnosis, but rapidly normalized with induction therapy.

Figure 4: BDG CSF associations with mortality. Kaplan-Meier survival curve showing 10-week survival from time of diagnosis in the 72 Ugandan patients with cryptococcal meningitis included in the analysis. Abbreviation: BDG, (1→3)-Beta-D-Glucan.