Delta-like 1 protein, Vitamin D Binding Protein, and Fetuin Measurement in Cerebrospinal Fluid for Detection of *Mycobacterium tuberculosis* meningitis

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Abstract

Background: Tuberculosis meningitis (TBM) diagnosis is notoriously difficult, and new biomarkers are needed for improved diagnostic accuracy. We evaluated the diagnostic utility of 3 novel biomarkers. 1). Delta-like ligand 1 (DLL1), a Notch ligand, which selectively drives antigen-specific CD4 T helper1 cell responses; 2) vitamin D binding protein, which transports vitamin D metabolites and may be involved in macrophage activation; 3) fetuin, which inhibits release of high mobility group box 1 from macrophages.
Methods: 139 HIV-infected persons with suspected meningitis were enrolled: 22 had TBM, 71 cryptococcal meningitis, 16 aseptic meningitis and 30 no meningitis. TBM was diagnosed either by GeneXpert MTB/Rif assay (Cepheid, Sunnyvale, CA) or Bactec MGIT culture. *Cryptococcus* was diagnosed by cryptococcal antigen lateral flow assay (IMMY Inc., Norman, OK). We measured CSF concentrations by ELISA of DLL1 (n=136), VDBP (n=130), and fetuin (n=130). 17 CSF cytokines and chemokines were also measured by Luminex multiplex-assay.
Results: Patients with TBM or aseptic meningitis had significantly higher total protein, WBC counts, DLL1, VDBP, and fetuin levels than patients with *Cryptococcus* or no meningitis. Receiver operator characteristic curves determined the area under the curve (AUC) for DLL1 of 0.789 (95% CI 0.676-0.902), VDBP of 0.794 (95% CI 0.701-0.887), and fetuin of 0.824 (0.739-0.909). Using logistic regression, DLL1 along with interleukin (IL)-1b and *Cryptococcus*-status provided 71% (15/21) sensitivity, 92% specificity (84/91), 68% (15/22) positive predictive value (PPV), and 93% (84/90) negative predictive value (NPV). Using *Cryptococcus*-status with IL-1b provided 81% (17/21) sensitivity, 95% (86/91) specificity, 77% (17/22) PPV, and 96% (86/90) NPV.
Conclusions: CSF DLL1, VDBP, and fetuin along with IL-1b may have roles as low cost adjunctive diagnostic tools for TBM. Misclassification bias (non-detection of TBM classified as aseptic meningitis) may limit this study, and larger studies are required in the future.

Background

•In settings where TB-HIV co-infection is common, TBM mortality is >50% with difficulty and delay in diagnosis common.

•Ziehl-Neelsen staining for acid-fast bacilli (AFB) is rapid but sensitivity is poor (usually 10-20%)

•Culture is more sensitive but slow growth (2-6 weeks depending on the media used) precludes rapid clinical decision-making and sensitivity is still only 60-70% sensitive.

•A rapid, accurate diagnostic method of detecting *Mycobacterium tuberculosis* in CSF would likely improve outcomes.

•Delta-like 1 protein (DLL1) is a trans-membrane protein and part of the Notch-ligand family and plays a role in fatty acid metabolism which is important for the *Mycobacterium tuberculosis* cell wall and;

•DLL1 has been found to selectively drive antigen-specific CD4 T helper1 cell responses and DLL1 polymorphisms increase susceptibility to other intracellular (Th1) organisms and may drive a similar Th1 response in patients infected with *Mycobacterium tuberculosis*.

•Vitamin D binding protein (VDBP) transports vitamin D metabolites and may increase leukocyte chemotatic activity of precursor peptides to macrophage activation factor - both of which may be important in immune response to *M tuberculosis* via increased macrophage activation.

•Fetuin inhibits high mobility group box one which is released from macrophages when stimulated by interferon gamma or tumor necrosis factor alpha which are associated with tuberculosis infection.

•VDBP and fetuin are markers of bovine tuberculosis.

Study Population:

•HIV infected individuals suspected to have meningitis in Kampala, Uganda.

•Reasarch Participants Screened as part of either:

- Cryptococcal Optimal ART Timing (COAT) trial (clinicaltrials.gov NCT01075152) or
- Adjunctive Sertraline for the Treatment of HIV-Associated Cryptococcal Meningitis (ASTRO-CM) trial (clinicaltrials.gov NCT01802385) .

Methods

- TB microbiologic diagnosis was obtained by automated PCR via the GeneXpert system using the Xpert MTB/Rif cartridge (Cepheid Inc, Sunnyvale, CA, USA) and/or by CSF Bactec MGIT culture.
- Participants or their surrogates provided written informed consent.
- Cryptococcal meningitis was diagnosed by cryptococcal antigen (CRAG) lateral flow assay
- Patients without evidence of cryptococcus or TBM were categorized as aseptic meningitis (protein > 100mg/dL or WBC count >10 cells/μL) or no meningitis (protein < 100mg/dL or WBC count <10 cells/μL)
- CSF biomarker concentrations were measured using the human soluble DLL1 ELISA kit (AdipoGen Inc., Incheon, Korea) and VDBP and Fetuin Elisa kits (R&D Systems Inc, Minneapolis MN) according to manufacturer instructions using cryopreserved specimens (-80°C).
- Luminex magnetic bead technology (Bio-Rad Laboratories, Hercules, CA, USA) analyzed 17 cytokines and chemokines according to manufacturer instructions using cryopreserved CSF (-80°C).
- Statistical analysis was done using SPSS version 21 (IBM Inc., Armonk, NY, USA).
- ANOVA and independent samples *t* tests were used to compare mean biomarker levels by diagnosis
- Area under the curve (AUC) was calculated using a receiver operator characteristic (ROC) curve.

Results

Table 1: Median (IQR) CSF concentrations of delta-like 1 protein (DLL1), vitamin D binding protein (VDBP), and Fetuin by diagnosis

CSF Biomarker Concentration	Tuberculous Meningitis	Cryptococcal Meningitis	Aseptic Meningitis	No Meningitis	P value
DLL1 , pg/mL	870 (558-1233)	422 (304-544)	671 (463-780)	233 (172-379)	<.001
VDBP, μg/mL	4.72 (2.97-8.96)	1.55 (0.74-2.71)	3.48 (1.30-9.11)	0.62 (0.34-1.10)	<.001
Fetuin, μg/mL	3.30 (2.19-4.55)	1.55 (0.82-2.37)	3.10 (1.42-4.24)	0.51 (0.29-0.76)	<.001

P values calculated from mean $\log_2$ transformed biomarker values analyzed by ANOVA.

Conclusions/Implications:

•CSF DLL1, VDBP, and Fetuin levels are significantly higher in patients with TB meningitis than in either cryptococcal meningitis or aseptic meningitis.

•DLL1, VDBP, and Fetuin show moderate accuracy in identifying microbiologic TB meningitis

•Combining DLL1 with Fetuin, WBC, and cryptococcal antigen improved accuracy in TBM prediction although less so than CrAg status in combination with IL1-β and IL-17 .

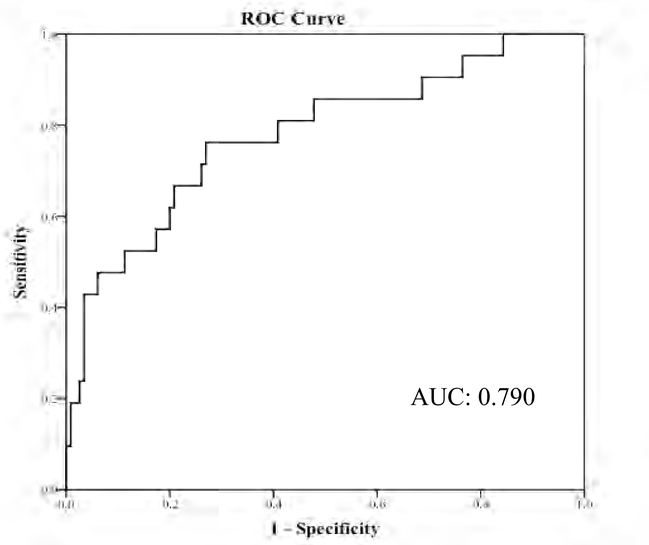
•These biomarkers may have roles as rapid (≤1 day) tests in the current ELISA form for TBM diagnosis

Table 2: Test performance for detection of TB meningitis

CSF Biomarkers	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
DLL1	32% (7/22)	98% (103/105)	78% (7/9)	87% (103/118)
VDBP	14% (3/22)	97% (102/105)	50% (3/6)	84% (102/121)
Fetuin	14% (3/22)	98% (103/105)	60% (3/5)	84% (103/122)
DLL1, Fetuin, WBC, Cryptococcal Antigen	64% (14/22)	93% (98/105)	67% (14/21)	93% (98/106)
IL1-β, IL-17, Cryptococcal Antigen	81% (17/21)	97% (88/91)	85% (`7/20)	96% (88/92)

IL = interleukin; VDBP = vitamin D binding protein

Figure 1: ROC curve to evaluate CSF DLL1 performance for TBM diagnosis



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