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Diagnostic Performance of a Multiplex PCR assay for Meningitis in an HIV-infected Population in Uganda

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Abstract

Background: Meningitis remains a worldwide problem, and rapid diagnosis is essential to optimize survival. Delay in diagnosis leads to excess morbidity, mortality and healthcare costs related to unnecessary empiric treatment and isolation procedures.

Methods: From January-May 2014, cerebrospinal fluid (CSF) from 49 HIV-infected persons with suspected meningitis in Kampala, Uganda was collected at time of diagnosis (n=23) and among persons with cryptococcal meningitis (CM) at therapeutic lumbar punctures (n=76). Standard bacterial, mycobacterial and fungal CSF diagnostics were performed on site. Cryopreserved CSF specimens (200 mcL) were then analyzed on the FilmArray™ System using a Meningitis/Encephalitis PCR panel (BioFire Diagnostics, Salt Lake City, UT; research use only). The panel targets 16 common pathogens: 6 bacterial, 8 viral, and Cryptococcus neoformans/gattii speciation. Operators were blinded to microbiology results. We assessed the diagnostic performance of the panel.

Results: The FilmArray™ multiplex PCR panel detected Cryptococcus in the CSF of all patients diagnosed with a first episode of cryptococcal meningitis by quantitative fungal cultures (n=18) with 100% sensitivity and specificity. In second episodes, the FilmArray™ system was able to differentiate between fungal relapse (n=3) vs paradoxical immune reconstitution syndrome (IRIS) and/or sterile cultures (n=5). In patients receiving antifungal therapy, FilmArray™ predicted follow up culture sterility with 71% negative predictive value. The first possible case of C. gattii meningitis in Uganda was detected. EBV was frequently detected in this HIV-infected population regardless of whether or not they had active cryptococcal infection [87% with (34/39) and 50% without (n=5/10) cryptococcosis]. Other pathogens detected included CMV (n=4), HHV-6 (n=3), HSV-2 (n=2), VZV (n=1), and Streptococcus pneumoniae (n=1).

Conclusion: The FilmArray™ multiplex PCR panel offers a promising platform for the rapid diagnosis of CNS infections. PCR testing appears to be particularly useful in cryptococcal disease, distinguishing species, predicting culture sterility, and differentiating IRIS from culture-positive cryptococcal relapse in patients with recurrent symptoms.

Background

- Meningitis remains a worldwide problem, particularly in the HIV-infected population.
- Rapid diagnosis of meningitis is essential to optimize survival and minimize healthcare costs related to unnecessary empiric treatment and isolation procedures.
- The Meningitis/Encephalitis FilmArray™ (BioFire Diagnostics, Salt Lake City, UT) is a multiplex PCR currently being developed for the detection of CNS pathogens in CSF.
- This panel is capable of rapidly (1 hour turn-around) targeting multiple pathogens with minimal volume (200 µL) and time (2-minutes hands-on) requirements.
- We evaluated the use of the FilmArray system in an HIV-infected Ugandan population.

Study Population

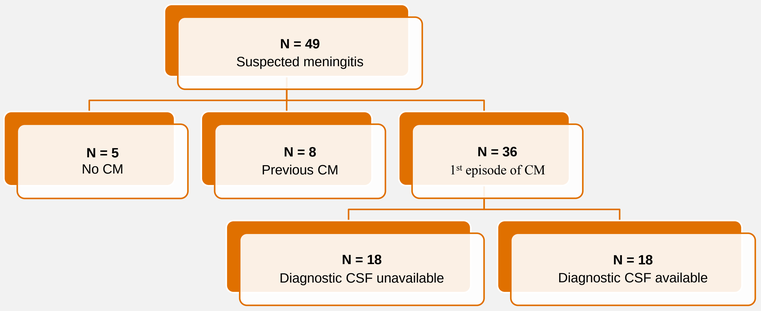

Figure 1: Analysis Cohort:

- 49 HIV-infected persons enrolled from May-June, 2014 as part of prospective cohort of hospitalized patients with suspected meningitis.
- 73% (36/49) of subjects presented with first episode of CM, diagnosed by CSF cryptococcal antigen (CRAG) and confirmed by CSF culture.
  - 18 subjects had diagnostic CSF specimens available for FilmArray testing.
  - 18 subjects did not have diagnostic specimens available for FilmArray testing.
  - CSF specimens collected 2-28 days after CM diagnosis was analyzed by PCR in 31/36 individuals with first episode of CM (Table 1B).
- 8 individuals with previous history of CM presenting with recurrent symptoms.
- 5 individuals with suspected meningitis that did not have evidence of CM.

Methods

- CSF was collected by lumbar puncture at diagnosis (n=23) or 3-20 days after CM diagnosis (n=76), stored at -80ºC and shipped on dry ice.
- Multiplex PCR was performed at BioFire, Inc. laboratories using the FilmArray™ Meningitis/Encephalitis panel (research use only) on cryopreserved, blinded specimens.

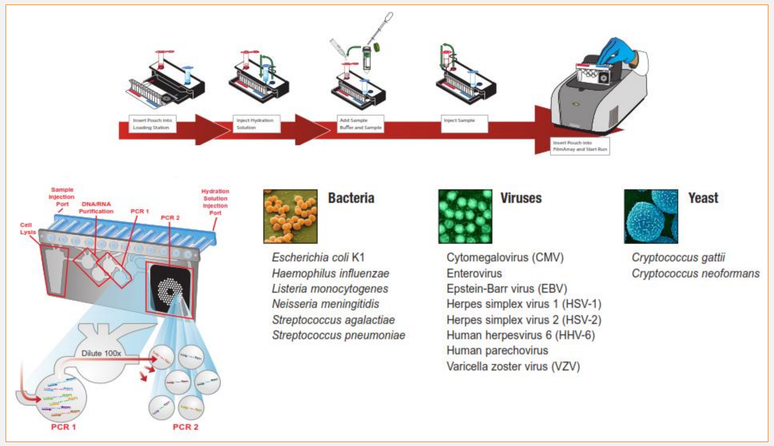

Figure 2: The FilmArray™ Multiplex PCR System. The system employs a reagent freeze-dried pouch that stores components necessary for sample preparation, reverse transcription, PCR and detection. The user injects hydration solution and sample combined with sample buffer into the pouch. A nested multiplex PCR is performed in a two-step process and, using endpoint melting curve analysis, a result is generated for each of 16 common pathogens.

- Diagnostic performance of multiplex PCR for CM was compared vs. reference standard of positive CSF cryptococcal culture.
- Additional analysis included:
  - The ability to identify cases of relapse in persons with a previous history of CM.
  - The value of PCR for predicting CSF culture sterility in persons treated for CM.
  - The occurrence of other CNS pathogens in this population of HIV-infected individuals with suspected meningitis in Uganda

Diagnostic Performance for CM

(A) Diagnostic specimens

		CSF Culture +	CSF Culture -	
FilmArray	+	18	0	PPV 100%
	-	0	5	NPV 100%
		Sensitivity 100%	Specificity 100%	

(B) Specimens obtained after diagnosis

		CSF Culture +	CSF Culture -	
FilmArray	+	39	9	PPV 81%
	-	8	20	NPV 71%
		Sensitivity 83%	Specificity 69%	

Table 1: Performance of multiplex PCR in CSF for cryptococcal meningitis. Performance characteristics of FilmArray compared to CSF cryptococcal culture in (A) diagnostic CSF specimens, and (B) CSF specimens obtained via therapeutic lumbar puncture in individuals receiving antifungal therapy after a diagnosis had already been established.

- The assay provided 100% sensitivity and specificity compared to cryptococcal cultures in diagnostic CSF specimens (n=23).
- In CSF specimens obtained from individuals already receiving antifungal therapy (n=76)
  - FilmArray predicted conversion to culture sterility with 71% negative predictive value.
  - This included CSF from individuals with both first (n=65 from 31 individuals) and second (n=11 from 8 individuals) episodes of CM.
- In 8 individuals with second episode of CM (symptomatic relapse), FilmArray was:
  - Positive for *Cryptococcus* in all cases of fungal relapse (n=3).
  - Negative for *Cryptococcus* in all cases of paradoxical immune reconstitution syndrome (IRIS) and/or sterile cultures (n=5).

Pathogen Detection in CSF

- In this population of advanced AIDS patients with suspected meningitis in Uganda, the FilmArray Meningitis/Encephalitis panel detected 8 distinct pathogens in CSF (**Figure 3**).
- *C. neoformans* was very common, as expected.
  - Unexpectedly, the system also detected one case of *C. gattii*
  - If confirmed, this would be the first documented case of *C. gattii* in Uganda
- In cases of CM, viral co-infection was extremely common, though the clinical significance of this is unknown (**Figure 4**).
  - EBV was frequently detected.
  - Other viruses detected include CMV, HHV-6, HSV-2, and VZV.
- Pneumococcus was detected in one culture-negative CSF specimen in a patient receiving empiric antibiotics.

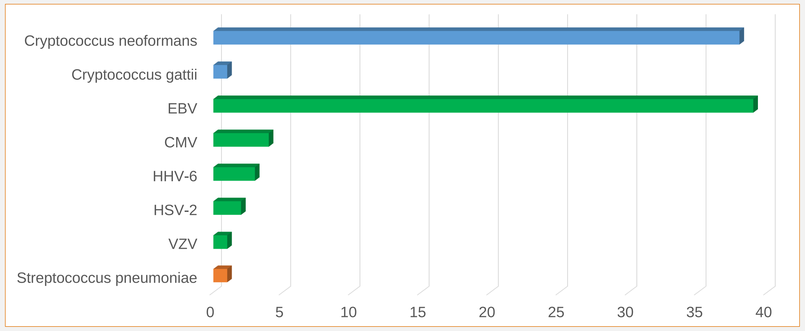

Figure 3: Frequency of pathogens Detected in CSF by FilmArray™ Meningitis/Encephalitis Multiplex PCR System. The system detected two species of *Cryptococcus* (blue), five viral pathogens (green) and one case of *Streptococcus pneumonia* (orange).

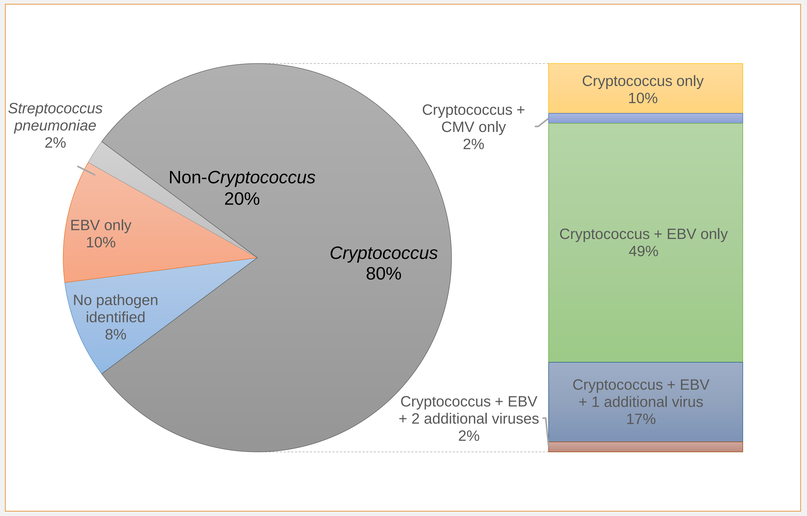

Figure 4: Distribution of Pathogens Detected in CSF and the Relationship to Other Potential Co-infections. Cases of CM were frequently associated with viral co-infection with EBV and other viruses.

Conclusions

- Multiplex PCR offers a promising platform for the rapid and accurate diagnosis of CNS infections, though the FilmArray system has not yet been evaluated by the FDA.
- A multiplex PCR capable of targeting additional pathogens in immune-compromised individuals (*M. tuberculosis,* JC virus, *Toxoplasma*) would be a significant advance.
- PCR testing appears to be useful in cryptococcal disease, distinguishing species, predicting culture sterility, and differentiating IRIS from culture-positive relapse.
- Additional studies are needed to validate the role and cost-effectiveness of multiplex PCR in the diagnosis and monitoring of CNS infections.