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. Cryptocapsid CSF specimens (200 mL) 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 species. 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=11) 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 (IRS) 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 [34.9] and 50% without [n=15] 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 IRS from culture-positive cryptococcal relapse in patients with recurrent symptoms.

Methods

- CSF was collected by lumbar puncture at diagnosis (n=23) or 3-20 days after CM diagnosis (n=76), stored at -80°C 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.

- 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.

Study Population

- N = 48 suspected meningitis
- N = 46 confirmed meningitis
- N = 36 CSF Culture
- N = 36 CSF Culture
- N = 10 Cryptococcus neoformans
- N = 10 EBV
- N = 10 CMV
- N = 10 HHV-6
- N = 10 HSV-2
- N = 10 VZV
- N = 10 Streptococcus pneumoniae

Diagnosis Performance for CM

- Diagnostic specimens: CSF Culture
- PPV 100% NPV 100%
- Sensitivity 100% Specificity 100%
- PPV 80% NPV 71%
- Sensitivity 60% Specificity 60%

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.
- 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 (IRS) and/or sterile cultures (n=5).

Pathogen Detection in CSF

- 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.

- 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.

Results:

- The FilmArray multiplex PCR platform performed well in terms of sensitivity and specificity.
- The system was able to differentiate between fungal relapse and paradoxic immune reconstitution syndrome.
- The assay provided 100% sensitivity and specificity compared to cryptococcal cultures in diagnostic CSF specimens.

Conclusion:

- 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 IRS 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.