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Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda

Joshua Rhein [a,b,*], Nathan C. Bahr [a], Andrew C. Hemmert [c], Joann L. Cloud [c], Satya Bellamkonda [c], Cody Oswald [c], Eric Lo [c], Henry Nabeta [b], Reuben Kiggundu [b], Andrew Akampurira [b], Abdu Musubire [a,b], Darlisha A. Williams [a,b], David B. Meya [a,b], David R. Boulware [a], on behalf of the ASTRO-CM Team [1]

[a] Division of Infectious Disease and International Health, Department of Medicine, University of Minnesota, Minneapolis, MN, USA
[b] Infectious Disease Institute, Makerere University, Kampala, Uganda
[c] BioFire Diagnostics, LLC, Salt Lake City, UT, USA

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ABSTRACT

Meningitis remains a worldwide problem, and rapid diagnosis is essential to optimize survival. We evaluated the utility of a multiplex PCR test in differentiating possible etiologies of meningitis. Cerebrospinal fluid (CSF) from 69 HIV-infected Ugandan adults with meningitis was collected at diagnosis (n = 51) and among persons with cryptococcal meningitis during therapeutic lumbar punctures (n = 68). Cryopreserved CSF specimens were analyzed with BioFire FilmArray® Meningitis/Encephalitis panel, which targets 17 pathogens. The panel detected *Cryptococcus* in the CSF of patients diagnosed with a first episode of cryptococcal meningitis by fungal culture with 100% sensitivity and specificity and differentiated between fungal relapse and paradoxical immune reconstitution inflammatory syndrome in recurrent episodes. A negative FilmArray result was predictive of CSF sterility on follow-up lumbar punctures for cryptococcal meningitis. EBV was frequently detected in this immunosuppressed population (n = 45). Other pathogens detected included: cytomegalovirus (n = 2), varicella zoster virus (n = 2), human herpes virus 6 (n = 1), and *Streptococcus pneumoniae* (n = 1). The FilmArray Meningitis/Encephalitis panel offers a promising platform for rapid meningitis diagnosis.

1. Introduction

Meningitis is a common clinical condition in sub-Saharan Africa. In the African meningitis belt, bacterial pathogens such as *Neisseria meningitidis* and *Streptococcus pneumoniae* have historically been among the most common etiologies, resulting in an estimated 800,000 cases between 1996 and 2010 (WHO, 2013). The higher prevalence of HIV infection in Eastern and Southern Africa has had a dramatic impact on the etiology of meningitis, with an emergence of *Cryptococcus neoformans* (Amuron et al., 2011; Bhagwan and Naidoo, 2011; Cohen et al., 2010; Durski et al., 2013; Hakim et al., 2000; Jarvis et al., 2010; Siddiqi et al., 2014) at an estimated 1 million cases of cryptococcal meningitis occurring in 2008 (Park et al., 2009). In immunocompromised hosts, the broad spectrum of potential diagnoses creates the need for multiplex assays to streamline diagnosis. Rapid diagnosis of meningitis is essential to optimize survival and minimize unnecessary healthcare costs related to isolation procedures and empiric treatment.

Additionally, as cryptococcal meningitis is now the most common cause of meningitis in Africa (Amuron et al., 2011; Bhagwan and Naidoo, 2011; Cohen et al., 2010; Durski et al., 2013; Hakim et al., 2000; Jarvis et al., 2010; Siddiqi et al., 2014), recurrent second episodes create a diagnostic dilemma of differentiating culture-positive relapse from paradoxical immune reconstitution inflammatory syndrome (IRIS) after initiating antiretroviral therapy. The gold standard to distinguish relapse from IRIS remains cerebrospinal fluid (CSF) culture, which can often take 5–14 days in the setting of culture-positive relapse. India ink is unhelpful in this setting, as unviable *Cryptococcus* are often still present in CSF with IRIS (Boulware et al., 2010). This delay in diagnosis creates a clinical problem. Persons with IRIS likely benefit from anti-inflammatory therapy (Bahr et al., 2013; Meintjes et al., 2010), yet the use of corticosteroids in persons with relapse may lead to detrimental outcomes (Musubire et al., 2013). Similarly, the treatment for relapse with amphotericin is associated with potentially severe toxicities and may not be of benefit for persons with paradoxical IRIS (Bahr et al., 2014).

This study aimed to comprehensively evaluate the utility of using a new multiplex PCR panel (FilmArrray Meningitis/Encephalitis panel; BioFire Diagnostics, LLC, Salt Lake City, UT, USA) in determining the microbiologic etiologies of meningitis in an HIV-infected adult population

* Corresponding author. Tel.: +1 612 624 9996; fax: +1 612 625 4410.
E-mail address: joshua.rhein@gmail.com (J. Rhein).

[1] ASTRO-CM Team members: Jane Francis Ndyetukira, Cynthia Ahimbisibwe, Florence Kugonza, Sruti S. Velamakanni, Alice Namudde, Alisat Sadiq, Tadeo Kiiza Kandole, Julian Kaboggoza, Eva Laker, Tony Luggya, Liliane Tugume, Elissa Butler, Jonathan Dyal, Julie Neborak, Lexie King, Nathan Yueh, Kathy Huppler Hullsiek, Ryan Halupnick, Paul R Bohjanen, Andrew Kambugu.

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with suspected meningitis in Kampala, Uganda. With a single PCR test, the FilmArray Meningitis/Encephalitis panel allows detection of several common pathogens (bacteria, viruses, and fungi) that cause meningitis, including *Cryptococcus*.

2. Materials and methods

2.1. Setting and patients

We conducted a prospective cohort study of 69 HIV-infected adult inpatients presenting with suspected meningitis between January 2014 and May 2014 at Mulago National Hospital, a tertiary referral hospital in Kampala, Uganda. This was conducted as part of a diagnostic substudy for the Adjunctive Sertraline for the Treatment of HIV-Associated Cryptococcal Meningitis (ASTRO-CM) pilot trial (ClinicalTrials.gov: NCT01802385). We only enrolled patients who presented with suspected meningitis and provided written informed consent for lumbar puncture (LP). If HIV serological status was unknown, rapid HIV testing was performed. Institutional review board approval occurred at all relevant organizations.

2.2. Routine CSF examination

A total of 119 CSF specimens were prospectively collected by LP at time of meningitis diagnosis (n = 51) or 2–28 days after cryptococcal meningitis diagnosis (n = 68) in the case of therapeutic LPs. The Makerere University Microbiology Laboratory performed CSF testing on the day of collection to determine white cell count and protein level. Cryptococcal meningitis was diagnosed immediately at the bedside using cryptococcal antigen (CRAG) lateral flow assay (IMMY; Norman, OK, USA). Quantitative CSF fungal culture was performed on fresh CSF in the Makerere Microbiology Laboratory with 5 1:10 serial dilutions of 100 μL of CSF, as previously described (Bicanic et al., 2007). Among persons with cryptococcal meningitis, therapeutic LPs were routinely performed using manometers on days 3, 7, 10, and 14 of amphotericin therapy and additionally as needed for intracranial pressure control. Quantitative CSF fungal cultures were performed with every CSF collection, with the exception of 1 specimen that had a positive culture without quantification. Among CSF specimens negative by CRAG, Gram stain, bacterial culture, and tuberculosis testing were performed.

2.3. FilmArray multiplex PCR system

With diagnostic or therapeutic LPs (specific for cryptococcal meningitis participants), a 1-mL aliquot of CSF was cryopreserved at −80 °C, as volume allowed. Cryopreserved CSF specimens were then shipped in dry ice to BioFire Diagnostics, where 200 μL of CSF was analyzed using the FilmArray Meningitis/Encephalitis system (Fig. 1). The system employs a reagent freeze-dried pouch that stores components necessary for sample preparation, reverse transcription, PCR, and detection (Babady, 2013; Ruggiero et al., 2014; Ward et al., 2015). The user injects hydration solution and sample combined with sample buffer into the pouch. Sample extraction, purification, and multiplexed-nested PCR are performed in the enclosed pouch with the FilmArray instrument. Using endpoint melting curve analysis, a result is generated for each of 16 targeted pathogens: 6 bacterial, 8 viral, and *Cryptococcus neoformans/gattii*. FilmArray operators were blinded to prior microbiology results.

CSF from study subjects with known tuberculosis, by culture or GeneXpert (Cepheid, Sunnyvale, CA, USA), which is part of the work-up for the main ASTRO-CM trial, were not included in this substudy due to biosafety concerns in the transport and analysis by FilmArray. An Investigation Use Only version of the FilmArray Meningitis/Encephalitis panel was used to test these samples. Performance characteristics of this FilmArray panel had not been evaluated by the Food and Drug Administration (FDA) at the time of this study. Confirmatory PCR testing was carried out using Argene® (bioMérieux, Marcy l'Etoile, France) for viral pathogens and genesig® *Streptococcus pneumoniae* PCR (Primerdesign, Southamptom, United Kingdom). Epstein-Barr virus (EBV) was not validated by additional confirmatory testing due to its ubiquitous nature and unclear significance in this immunocompromised population, as described previously (Rajasingham et al., 2015).

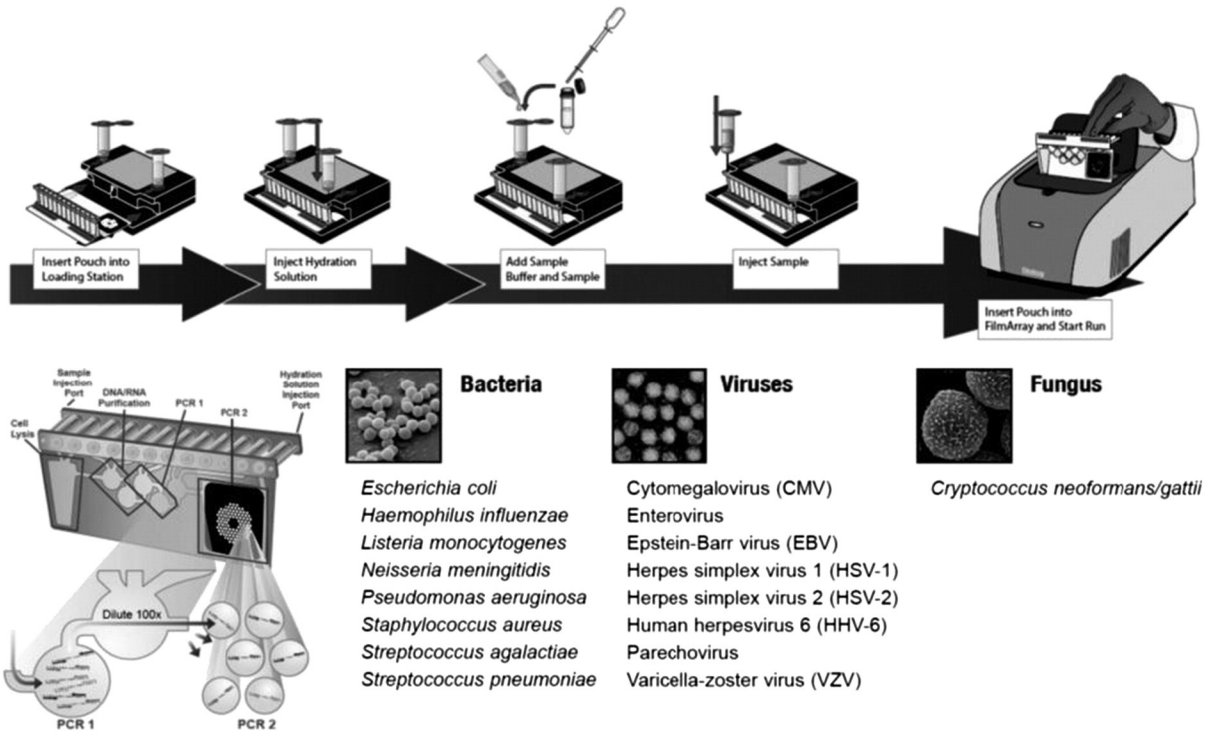

Fig. 1. The FilmArray multiplex PCR system.

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