Open Forum Infectious Diseases

BRIEF REPORT

Delayed Diagnosis of Tuberculous Meningitis Misdiagnosed as Herpes Simplex Virus-1 Encephalitis With the FilmArray Syndromic Polymerase Chain Reaction Panel

Carlos A. Gomez,[1,2] Benjamin A. Pinsky,[1,2] Anne Liu,[1,3] and Niaz Banaei[1,2,4]

[1]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, [2]Department of Pathology, and [3]Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, Stanford University School of Medicine, California; [4]Clinical Microbiology Laboratory, Stanford University Medical Center, Palo Alto, California

The FilmArray meningitis/encephalitis (ME) panel is a novel syndromic, nucleic acid amplification test for diagnosis of acute meningitis and encephalitis. Emerging data on its performance are concerning for false-positive results. We present a case of tuberculous meningitis misdiagnosed as herpes simplex virus-1 encephalitis with the FilmArray ME panel. Strategies to mitigate erroneous results are discussed.

Keywords. encephalitis; FilmArray panel; meningitis; molecular diagnostics; syndromic panel.

Syndromic nucleic acid amplification test panels are multiplex assays that simultaneously detect a broad range of pathogens directly from clinical specimens. Syndromic panels have advanced the diagnosis of infectious diseases by reducing turnaround times, simplifying laboratory workflow, guiding antimicrobial therapy, and improving infection prevention practices. In recent years, several syndromic panels have been cleared by the US Food and Drug Administration (FDA) for the diagnosis of respiratory and gastrointestinal infections.

In October 2015, the FDA cleared the first multiplex, meningitis/encephalitis (ME) panel (FilmArray ME panel; BioFire Diagnostics LLC, Salt Lake City, UT) for the diagnosis of most common infectious etiologies of acute central nervous system (CNS) infections. This fully automated, sample-to-answer, multiplex polymerase chain reaction (PCR) assay requires <2 minutes of hands-on time, and in 1 hour it tests for 14 ME pathogens, including bacteria, fungi, and viruses [1]. Although the FilmArray ME panel is attractive, as with any new test, healthcare providers should be familiar with its performance characteristics and limitations, particularly false-positive results. The latter may not only result in needless therapy with potential drug toxicity, but in the context of an immunocompromised host, false-positive results may halt further diagnostic efforts aimed at broadening the differential diagnosis, which may delay life-saving therapy.

In this study, we present a case of tuberculous meningitis leading to severe neurological sequelae, in an immunocompromised patient whose diagnosis was delayed due to a false-positive herpes simplex virus (HSV)-1 result with the FilmArray ME panel. Using lessons learned from this case and findings from the largest trial conducted to date, we discuss clinical and laboratory strategies to minimize false results.

CASE PRESENTATION

A 75-year-old Vietnamese man, who immigrated to California more than 40 years ago, was admitted to the emergency department at a community hospital after he was noted to have gradual-onset confusion and speech difficulties for 2 weeks. Ten months ago, the patient was diagnosed with follicular lymphoma and underwent 6 cycles of bendamustine and rituximab leading to complete remission. His last cycle of chemotherapy was completed 3 months before the current admission. Upon arrival, he seemed confused, disoriented to time and place, but without focal neurological deficits. A noncontrast brain magnetic resonance imaging (MRI) revealed no significant abnormalities. Lumbar puncture cerebrospinal fluid (CSF) revealed a neutrophilic-predominant pleocytosis (white blood cell count [WBC] 210 cells/μL, 72% neutrophils), glucose 67 mg/dL, and protein 587 mg/dL. Cerebrospinal fluid tested with the FilmArray ME panel was positive for HSV-1, prompting the initiation of intravenous acyclovir therapy for HSV encephalitis. Routine cultures of CSF and blood were reported as no growth. Confirmatory PCR testing for HSV-1 was not performed. After 7 days of antiviral therapy, the patient's mental status continued to decline. A repeat brain MRI showed the development of transependymal flow of CSF into the periventricular white matter, a radiographic finding consistent with evolving hydrocephalus. Ten days after hospital admission, the patient was transferred to our institution for his care.

Upon arrival, the patient was sedated, intubated with appropriately reactive pupil reflexes. The remainder of the physical examination was unremarkable. A repeat lumbar puncture showed increased opening pressure (35 cm H_2O), pleocytosis (WBC 99 cells/μL, 56% lymphocytes, 43% neutrophils), glucose 39 mg/dL, and protein 321 mg/dL. Cerebrospinal fluid Gram, calcofluor-white, and acid-fast bacilli stains were all negative. A repeat

Received 19 September 2016; editorial decision 7 November 2016; accepted 17 November 2016.
Correspondence: N. Banaei, MD, Stanford Health Care, Clinical Microbiology Laboratory, 3375 Hillview Ave, Rm. 1602, Palo Alto, CA 94304 (nbanaei@stanford.edu).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofw245

contrasted MRI of the brain and full spine showed diffuse leptomeningeal enhancement compromising the basal meninges (brainstem, cerebellar folia, and trigeminal nerves) as well as along the entire spinal cord including the conus medullaris and cauda equine. An electroencephalogram showed severe-diffuse slowing pattern without seizure activity, compatible with global encephalopathy. An external ventricular drain was placed on hospital day 5 to relieve intracranial hypertension. Because of acyclovir treatment failure, an Infectious Diseases (ID) consult was requested to expand the diagnostic workup for chronic meningitis. Per the ID recommendation, additional CSF testing by real-time PCR for HSV-1/HSV-2 (artus HSV-1/2 QS-RGQ Kit; QIAGEN, Germantown, MD), VZV, and CMV (also from QIAGEN), and cryptococcal antigen detection by lateral flow immunochromatography (IMMY, Norman, OK) all yielded negative results. On hospital day 7, *Mycobacterium tuberculosis* nucleic acid testing on a CSF sample, using a laboratory-developed PCR assay [2], was positive and tuberculosis therapy with first-line drugs (isoniazid, rifampin, pyrazinamide, and ethambutol) and dexamethasone were initiated. Cerebrospinal fluid cultured in liquid medium (MGIT960 system; Becton Dickinson, Franklin Lakes, NJ) turned positive for *M tuberculosis* after 13 days. Phenotypic susceptibility testing with first-line drugs demonstrated a pan-susceptible isolate. Unfortunately, over the following weeks, despite aggressive clinical management, the patient did not have meaningful neurologic recovery and eventually required a tracheostomy and gastric feeding tube for transition to a rehabilitation ward. At the time of writing this report, he continued on tuberculosis therapy with severe neurological deficit.

To further investigate the FilmArray ME panel result, our microbiology laboratory retrieved and performed additional testing on the leftover CSF sample obtained at the community hospital. First, repeat testing with the FilmArray ME panel was negative for all targets. The HSV-1 real-time PCR, as described above, was also negative. Lastly, *M tuberculosis* PCR was positive.

DISCUSSION

Infectious meningitis and encephalitis represent healthcare emergencies requiring timely diagnosis and rapid initiation of effective antimicrobial therapy. Considering the potential impact of FilmArray ME panel on individualized care, infection control practices, and antimicrobial stewardship, its uptake is occurring in academic and community hospitals and is likely to increase in the future. Nonetheless, as illustrated by the case presented here, the performance characteristics of the FilmArray ME panel and ways to mitigate false results must be carefully considered before implementing it for use in routine clinical practice.

The diagnostic performance of the FilmArray ME panel was reported by Leber et al [3] in a well-designed prospective study, evaluating 1560 remnant CSF samples obtained as part of routine medical care, across 11 different sites in the United States. For the purposes of estimating analytical sensitivity and specificity, conventional CSF culture for bacterial pathogens and real-time PCR assays for viral agents and *Cryptococcus* spp were used as comparators. Discrepancies were evaluated by additional testing (when available) using alternative methods and blinded assessment of clinical, epidemiological, and laboratory data. Despite best intentions, this trial had some limitations and highlighted concerning findings. First, the overall positivity rate was low after resolution of discrepancies (7.6%, 119 of 1560) with 10 of 14 targets having less than 10 cases, with *Listeria monocytogenes*, *Streptococcus agalactiae*, and *Neisseria meningitidis* having zero confirmed cases. Therefore, despite 100% analytical sensitivity for 9 of 14 analytes—*Streptococcus pneumoniae* (4 of 4), *Escherichia coli K1* (2 of 2), *Haemophilus influenzae* (1 of 1), cytomegalovirus (3 of 3), HSV-1 (2 of 2), HSV-2 (10 of 10), human parechovirus (9 of 9), *varizella zoster-virus* (4 of 4), and *Cryptococcus neoformans/Cryptococcus gattii* (1 of 1)—a larger validation study is warranted to estimate the sensitivity of FilmArray ME panel for these pathogens. Second, most relevant to our case report, false-positive results accounted for 15.6% (22 of 141) of all positive results with the FilmArray ME panel. False-positive results accounted for 41% (9 of 22) of bacterial, 9.6% (11 of 114) of viral, and 40% (2 of 5) of *Cryptococcus* spp results. As illustrated in Table 1, the positive predictive value of the FilmArray ME panel ranged from 50% to 100% for individual targets.

In regard to HSV-1 detection, the comparator PCR assay did not confirm half of the HSV-1-positive results (2 of 4) detected by the FilmArray ME panel. The authors hypothesize that preanalytical sample contamination (eg, during sample collection) and/or detection of latent or reactivated *herpesviridae* in cells

Table 1. Positive Predicted Value of the FilmArray ME Panel*

Analyte	Confirmed Positives/Total Positives (%)
Streptococcus pneumoniae	9 of 16 (56)
Haemophilus influenzae	2 of 2 (100)
Streptococcus agalactiae†	No positives
Escherichia coli K1	2 of 3 (66)
Listeria monocytogenes†	No positives
Neisseria meningitidis†	No positives
HSV-1	2 of 4 (50)
HSV-2	11 of 12 (92)
CMV	4 of 6 (66)
VZV	6 of 7 (86)
HPeV	12 of 12 (100)
HHV-6	19 of 22 (79)
EV	49 of 51 (96)
Cryptococcus spp	3 of 5 (60)

Abbreviations: CMV, cytomegalovirus; EV, enterovirus; HHV, human herpes virus; HPeV, human parechovirus; HSV, herpes simplex virus; ME, meningitis/encephalitis; VZV, varizella zoster-virus.

*Adapted from reference [3].

†No confirmed cases of *S agalactiae, N meningitiditis,* or *L monocytogenes* were reported.

present in the CSF may explain the unexpectedly high rate of unconfirmed results among the *herpesviruses*. However, it is unlikely that these HSV results are due to particularly sensitive analytical performance characteristics because the FilmArray ME panel showed a higher lower-limit-of-detection for HSV-1 (1500 copies/mL [250 $TCID_{50}$/mL]) compared with the FDA-cleared Simplexa HSV-1&2 Direct Assay (Focus Diagnostics, Cypress, CA) (30 copies/mL [5 $TCID_{50}$/mL]) [3–5]. Moreover, studies comparing the performance of the Simplexa assay with laboratory-developed real-time PCR assays have shown excellent consensus agreement (>98%), without the identification of a disproportionate number of false-positive results [4, 5].

In light of the findings by Leber et al [3], the case presented here underscores the need for clinical and laboratory measures to minimize false-positive results with the FilmArray ME panel. A list of recommendations is summarized in Table 2. First, strict precautions should be followed to avoid contaminating the CSF sample during collection and laboratory processing. Second, false-positive results may be reduced by restricting testing to patients with high suspicion for acute-onset community-acquired CNS infection. Patients with suspicion for nosocomial or postsurgical meningitis should be excluded from testing because their causative pathogens differ from those in the FilmArray ME panel. The laboratory could enforce testing criteria based on presence of abnormal CSF cellular, glucose, and protein indices; however, this approach requires case-by-case consideration because HSV-2, enterovirus, and partially treated bacterial meningitis can present with normal cell count indices [6–8]. Third, positive bacterial and *Cryptococcus* results should be correlated with Gram stain results, CSF culture, and clinical findings; discrepant results could be held pending further investigation. Lastly, false positives can be avoided by performing confirmatory testing using conventional methods [9]. This can be most effective if laboratories automatically reflexed to confirmatory testing. For instance, positive results for bacterial analytes should be correlated with Gram stain and culture results, which should always be ordered in addition to the FilmArray ME panel. For viral and *Cryptococcus* analytes, confirmation should be pursued using (1) targeted-PCR and (2) cryptococcal antigen test and fungal culture, respectively. Alternatively, repeating testing with the FilmArray ME panel may be performed on positive samples. However, repeating may lead to discrepancies due to sampling error in specimens with low pathogen burden. Any discordant and/or unexpected result should be discussed between laboratory and ordering clinician. Other measures to maximize appropriate test interpretation and patient care with the FilmArray ME panel include describing test performance characteristics, diagnostic limitations, and the need for confirmatory testing during electronic order entry and result reporting. Although these measures add more labor and cost, they are necessary to ensure accurate diagnosis until more data become available to guide best clinical practice with this assay. Negative FilmArray ME panel results in patients with high-pretest probability of community-acquired infectious ME should also be confirmed with independent assays. This may be particularly important in immunocompromised patients (eg, transplant recipients) for which FilmArray performance data are currently lacking, and empiric therapy may be appropriate while awaiting further testing [10, 11].

Table 2. Clinical and Laboratory Measures to Mitigate False-Positive FilmArray ME Panel Results

Category	Measures
Preanalytical	
	• Inform clinician during electronic order entry about the intended patient population (ie, community-acquired acute meningitis/encephalitis), assay performance characteristics, limitations, and need for confirmatory testing with positive results • Inform clinician to use a face shield and proper CSF handling when performing lumbar puncture • Enforce testing criteria based on abnormal CSF indices (cell count, glucose, and protein) • Exclude patients with postsurgical meningitis (eg, status postcraniotomy, external ventricular devices, VP shunts)
Analytical	
	• Use dedicated biosafety cabinet for sample processing and pouch loading • Clean working area with bleach or equivalent before sample processing and between sample testing • Change gloves before handling each sample • Process one CSF sample and handle one pouch at a time
Postanalytical	
	• Correlate positive results with CSF Gram stain, and CSF indices (cell count, glucose, and protein) • Laboratories should hold results with discrepant findings until further investigation/confirmation • Confirm positive results with routine culture, targeted viral PCR assays, and *Cryptococcus* antigen testing; bacterial or fungal PCR amplicon sequencing may be indicated for culture-negative ME cases • Discuss discordant results with ordering clinician • Inform clinician electronically about assay performance characteristics, limitations, and need for confirmatory testing of positive results

Abbreviations: CSF, cerebrospinal fluid; ME, meningitis/encephalitis; PCR, polymerase chain reaction; VP, ventriculoperitoneal.

CONCLUSIONS

In summary, the FilmArray ME panel is being adopted for diagnosis of acute ME due to its ease of use, rapid turnaround time, and comprehensive panel. However, false-positive results are not uncommon and can have detrimental consequences on patient outcomes. Until sufficient data becomes available on the performance of the FilmArray ME in different patient populations, clinical and laboratory measures must be in place to mitigate false results.

Acknowledgments

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. U.S. Food & Drug Administration. FDA News Release. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm466360.htm. Accessed 24 August 2016.
2. Luo RF, Scahill MD, Banaei N. Comparison of single-copy and multicopy real-time PCR targets for detection of *Mycobacterium tuberculosis* in paraffin-embedded tissue. J Clin Microbiol **2010**; 48:2569–70.
3. Leber AL, Everhart K, Balada-Llasat JM, et al. Multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. J Clin Microbiol **2016**; 54:2251–61.
4. Heaton PR, Espy MJ, Binnicker MJ. Evaluation of 2 multiplex real-time PCR assays for the detection of HSV-1/2 and Varicella Zoster virus directly from clinical samples. Diagn Microbiol Infect Dis **2015**; 81:169–70.
5. Kuypers J, Boughton G, Chung J, et al. Comparison of the Simplexa HSV1 & 2 Direct kit and laboratory-developed real-time PCR assays for herpes simplex virus detection. J Clin Virol **2015**; 62:103–5.
6. Graber JJ, Rosenblum MK, DeAngelis LM. Herpes simplex encephalitis in patients with cancer. J Neurooncol **2011**; 105:415–21.
7. Henquell C, Chambon M, Bailly JL, et al. Prospective analysis of 61 cases of enteroviral meningitis: interest of systematic genome detection in cerebrospinal fluid irrespective of cytologic examination results. J Clin Virol **2001**; 21:29–35.
8. Seiden JA, Zorc JJ, Hodinka RL, Shah SS. Lack of cerebrospinal fluid pleocytosis in young infants with enterovirus infections of the central nervous system. Pediatr Emerg Care **2010**; 26:77–81.
9. Hanson KE, Couturier MR. Multiplexed molecular diagnostics for respiratory, gastrointestinal, and central nervous system infections. Clin Infect Dis **2016**; 63:1361–7.
10. DeBiasi RL, Kleinschmidt-DeMasters BK, Weinberg A, Tyler KL. Use of PCR for the diagnosis of herpesvirus infections of the central nervous system. J Clin Virol **2002**; 25(Suppl 1):S5–11.
11. Tunkel AR, Glaser CA, Bloch KC, et al. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis **2008**; 47:303–27.