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## Pitfalls Associated With the Use of Molecular Diagnostic Panels in the Diagnosis of Cryptococcal Meningitis

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We report the case of a kidney transplantation patient on chronic immunosuppressive therapy presenting with subacute meningitis. The final diagnosis of cryptococcal meningitis was delayed due to 2 false-negative cryptococcal results on a molecular diagnostic panel. Caution with such platforms in suspected cryptococcal meningitis is needed.

**Keywords.** cryptococcal meningitis; encephalitis; meningitis; molecular diagnostic techniques; polymerase chain reaction.

Worldwide, *Cryptococcus* is the most common cause of fungal meningitis [1]. Delayed diagnosis of cryptococcal meningitis is associated with increased morbidity and mortality [2, 3]. Culture remains the gold standard for diagnosis of cryptococcal disease [4]. The limitations of culture are that it can be time consuming and labor intensive. Cryptococcal antigen (CrAg) lateral flow assays are rapid, specific, and may be more sensitive than culture when the burden of organism is low [4]. However, in patients presenting with symptoms of meningo-encephalitis, the causative organism is frequently not clinically apparent, there can be a large potential differential diagnosis requiring consideration, and there may be limited available cerebral spinal fluid (CSF) for testing. Efforts have been made in recent years to develop assays that would allow testing for a wide variety of pathogens associated with meningo-encephalitis in a short time frame and using small volumes of CSF. The FilmArray Meningitis/Encephalitis (ME) Panel (BioFire Diagnostics, Salt Lake City, UT) uses multiplex polymerase chain reaction (PCR) to test for 14 targets using just 200  $\mu$ L of CSF with a reported

hands-on time of 2 minutes and a turnaround time of approximately 1 hour [5]. As with all diagnostic tools, it is paramount that clinicians interpret results from PCR panels such as these in the context of the patient's clinical presentation, and that they are aware of any pitfalls associated with such tests. Herein we report a case where the clinical outcome for the patient was negatively impacted by the use of a PCR panel.

### CASE REPORT

A 54-year-old woman sought medical attention for a headache of 4 days' duration, associated with nausea but without focal neurological signs. She had undergone deceased donor kidney transplantation for lupus nephritis-induced end-stage kidney disease 5 years previously and was receiving tacrolimus, mycophenolate mofetil (MMF), and prednisolone as immunosuppressive therapy. She was diagnosed with migraine and prescribed a short course of hydrocodone. She did not have a past history of migraines. Over the proceeding month, she presented with persistent headache twice more to her primary care provider, and on 3 occasions to the emergency department where she underwent computed tomography and magnetic resonance imaging (MRI) of the brain, both of which were within normal limits.

Two months after the headache onset, the patient presented to her local hospital with headaches, nausea and vomiting, and fever. She was commenced on broad-spectrum antimicrobial therapy. Her CSF revealed a white cell count (WCC) of 145 cells/cm<sup>3</sup> (27% neutrophils, 63% lymphocytes, 10% monocytes), glucose of 36 mg/dl, and protein of 72 mg/dl. Gram stain and routine bacterial cultures were negative. Fungal and mycobacterial cultures were not performed. A FilmArray Meningitis/Encephalitis (ME) Panel detected human herpes virus 6 (HHV-6) but was negative for the remaining 13 components tested for by the panel (*Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, cytomegalovirus, enterovirus, herpes simplex virus 1 and 2, human parechovirus, varicella zoster virus, and *Cryptococcus neoformans/gattii*). Antimicrobial therapy was discontinued, and the patient was discharged on analgesia with symptoms attributed to HHV6 infection.

In the weeks that followed, the patient continued to clinically deteriorate, developing difficulty with her gait and fecal incontinence. New onset of abdominal pain, now 3 months after the headache onset, prompted her to once again seek medical assessment. The patient was readmitted, and her work-up revealed acute pancreatitis of unknown etiology, with a lipase of 18 800 units/L. At this time, MMF was held and she continued on tacrolimus and prednisolone immunosuppression. On day

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11 of her admission, the patient became febrile to 38.6°C. Blood cultures were obtained, and she was initiated on empiric meropenem; subsequently, linezolid and micafungin were added. On day 19, after a fall, she was noted to be encephalopathic with psychomotor slowing, which triggered a repeat neurological workup. A brain MRI did not reveal any acute intracranial processes. Lumbar puncture (LP) revealed a WCC count of 320 cells/cm<sup>3</sup> (58% neutrophils, 37% lymphocytes), a glucose of 8 mg/dl, and protein of 121 mg/dl. No opening pressure was recorded. Again, a FilmArray ME Panel was performed, but it was negative for all pathogens included in the panel. CSF bacterial culture was negative.

On hospital day 20, the patient was transferred to a tertiary care academic center. On arrival, she was afebrile but remained encephalopathic, with altered speech. She intermittently followed simple commands and had evidence of meningismus. A high-volume (10 mL of CSF) LP was performed, and the IMMY cryptococcal antigen lateral flow assay was positive at a titer of 1:20. Her serum CrAg was also positive at 1:10. The patient was initiated on liposomal amphotericin and flucytosine for management of cryptococcal meningitis. CSF from the LP performed prior to transfer was obtained and was also found to be positive at a titer of 1:40. All cultures, including blood and CSF, were negative. The patient's symptoms improved on liposomal amphotericin and flucytosine, and she was ultimately discharged 3 weeks later on fluconazole.

## DISCUSSION

This case highlights some important issues associated with the use of PCR panels such as the FilmArray ME Panel in clinical practice. The clinical assay performance of the FilmArray ME Panel was assessed in a multicenter study that examined 1560 residual CSF samples from patients undergoing lumbar puncture at 11 sites across the United States [6]. In this study, the results from the FilmArray ME Panel were compared with what was considered by the study team to be an appropriate comparator method. The comparator was CSF culture for the bacteria and bidirectional PCR sequencing for the viruses and fungi included in the panel. The sensitivity for *Cryptococcus neoformans/gattii* was reported at 100% (1/1) and was included in the overall assay sensitivity despite being calculated from a single positive specimen. Specificity for *Cryptococcus* spp. was reported as 99.7% (1555/1559). Of note, the comparator method for *Cryptococcus* in this study was PCR and not culture or CrAg testing. The investigators reviewed the records of the 4 patients who had positive results by the FilmArray ME Panel but negative results by the comparator PCR and determined that 2 were falsely negative by the comparator, and thus true positives detected by the FilmArray ME Panel. Not included in the published results but included in the FilmArray ME Panel package insert were comparisons with the test results of the clinical labs. The FilmArray ME Panel was positive for 1 of the

8 (12.5%) specimens positive for CrAg, and positive for 2 of 3 (66.7%) that were culture positive. Of the 7 that were positive for CrAg but negative by the FilmArray ME Panel, all patients were on antifungals at the time of specimen collection or had a history of Cryptococcal meningitis.

Interestingly, a smaller preclinical study of a 16-target FilmArray ME Panel highlighted the importance of an appropriate comparator test [7]. When the 16-target assay was performed on 342 stored CSF specimens, 8 of 14 (57%) CrAg-positive specimens were positive by the FilmArray ME panel, with 1 specimen that was CrAg negative/FilmArray positive also testing positive by sequencing. The false-negative results came from specimens with relatively low CrAg titers and/or high PCR crossing thresholds, and therefore were likely related to low burden of disease. This may also explain why the FilmArray ME Panel performed well in subjects with first presentations of cryptococcal meningitis in a study performed in sub-Saharan Africa where the median quantitative culture was 8950 CFU/mL (IQR, 118–113 500 CFU/mL) [8]. Decreased sensitivity was observed in this study on follow-up CSF specimens obtained from therapeutic lumbar puncture in patients receiving appropriate antifungal therapy and presumably having lower fungal burdens. In those with positive cryptococcal cultures and quantitative colony count <100 CFU/mL, the sensitivity was 50% (6/12).

In the case reported herein, the patient was treated with the calcineurin inhibitor tacrolimus, a class of drugs known to have in vitro antifungal activity. In solid organ transplant recipients, tacrolimus has previously been associated with lower mortality rates in the setting of cryptococcal infection [9]. It is therefore possible that tacrolimus therapy may have contributed to the lower CrAg titers in this case, and potentially to the false-negative cryptococcal result obtained on the FilmArray ME Panel, although there are no specific data available in relation to the latter. It is also noteworthy that while echinocandins generally have little activity against *Cryptococcus neoformans* alone, in vitro synergy between caspofungin and tacrolimus has been reported [10].

To our knowledge, this is the first reported case of a patient whose clinical outcome was negatively impacted by a false-negative cryptococcal PCR on the FilmArray ME panel. The patient had known risk factors for cryptococcal meningitis with a typical clinical presentation and CSF profile, which ought to have prompted a more thorough investigation. This case serves to highlight to clinicians the importance of understanding the diagnostic accuracy of tests performed in order to ensure that they are appropriately interpreted, particularly in the setting where a sensitive, cheap, and easily performed alternative diagnostic test exists. Compared with culture and CrAg, the sensitivity of the FilmArray ME Panel for *Cryptococcus* in the United States appears to be in the mid-60% range at best. Patients with a low burden of disease or who are already on antifungals are

more likely to have a false-negative FilmArray ME Panel for *Cryptococcus*. This case also highlights the potential downside of testing for a multitude of pathogens, several of which may not be clinically relevant. In this case, the initial FilmArray ME panel performed was positive for HHV6, and the patient symptoms and abnormal CSF profile were initially inappropriately attributed to this, and possibly contributed to the delay in the diagnosis of this patient's cryptococcal meningitis.

In conclusion, clinicians should be aware that a negative cryptococcal result on this platform does not rule out cryptococcal meningitis, and standard assessment with CrAg and culture should be performed when the clinical scenario is consistent with cryptococcal infection.

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Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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