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Case report

Neisseria meningitidis and cytomegalovirus simultaneous detection in the filmarray meningitis/encephalitis panel and its clinical relevance

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ABSTRACT

A BioFire FilmArray Meningitis/Encephalitis test was performed on a 7-month old child suspected for bacterial meningitis. Two pathogens were detected, *Neisseria meningitidis* and cytomegalovirus (CMV). We verified the filmarray meningitis/encephalitis test by pan-bacterial assay to test for *Neisseria meningitidis* and CMV viral load test for the CMV detection. Pan-bacterial confirmed presence of *N. meningitidis*, but CMV was not detected by the CMV viral load test. Together with the manifestations of high fever, vomiting, diffuse petechial rash, bulging fontanel, and leukocytosis, it is a clear case of meningococcal meningitis, while CMV detection had no clinical relevance.

Introduction

Meningococci are gram–negative aerobic diplococci. *Neisseria meningitidis* can cause two life threatening diseases: meningococcal meningitis and fulminant septicemia (meningococcemia), which often occur together. The incubation period averages three to four days (range one to ten days) during which the individual is infectious. Meningococci colonize the upper respiratory tract and nonciliated mucosal cells internalize them. They traverse the mucosal cells, enter the submucosa, and in approximately 10–20% cases make their way to the bloodstream [1]. The bacteria may seed local sites such as the meninges, joints or the pericardium or multiply in the bloodstream, causing meningococcemia with petechiae [1,2]. Cytomegalovirus (CMV) is a double-stranded DNA virus that belongs to subfamily of Beta-herpesvirinae [3]. In children, CMV infection ranges from asymptomatic or mild disease in immunologically normal hosts, to severe and potentially life-threatening disease in newborns and immunocompromised children [4]. CMV infection of the central nervous system is well documented [5], mainly in immunodeficient patients [6].

Case report

A previously healthy 7-month old male infant was admitted due to fever of 40 °C for 2 days, and on the day of admission, vomiting and a rash. On physical examination, the infant had a fever of 39.3 °C, pulse 172/min and blood pressure of 102/53 mmHg. He was apathic, had diffuse petechial rash, and a bulging fontanel with no nuchal rigidity. Blood count showed leukocytosis of 22,740/mm^3 with a differential of 53.2% neutrophils and 33.9% lymphocytes, serum glucose 86 mg/dL and C-reactive protein (CRP) of 197.7 mg/L. Blood culture was obtained. Because of his condition and high suspicion of a bacterial infection, IV antimicrobials (ceftriaxone and vancomycin), dexamethasone and fluids were immediately started. Lumbar puncture was performed an hour later and showed a cerebrospinal fluid (CSF) with a protein of 82.5 mg/dL, glucose 47 mg/dL, and 1513 leukocytes with 75% neutrophils. Blood and CSF cultures were negative, as was CSF PCR for enteroviruses.

Because CSF culture was obtained after initiating IV antimicrobials and due to the clinical importance, a BioFire FilmArray Meningitis/Encephalitis test was performed in the CSF (Biomériuex). The test resulted positive for two pathogens, *N. meningitidis* and CMV. To confirm the causative pathogen(s), we further analyzed the CSF for CMV viral load [7] The PCR assay was done using RealStar® CMV PCR kit 1.0 (Altona Diagnostics), where the manufacture's limit of detection is 0.668 IU/μL (95%) and confidence interval of 95% is 0.323–2.258 IU/mL. Limit of detection in the BioFire FilmArray Meningitis/Encephalitis test is 4.3 IU/μL. CMV was tested only on CSF, and the result for the viral load PCR assay was "not detected". We further examined remnant of the infant serum at admission for CMV serology, and detected a positive IgG and a negative IgM CMV antibodies.

CSF was also studied by 16S sequencing [8]. For bacterial meningitis verification, we used 16S sequencing (a method often

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called "pan bacterial"). The method [8] is based on amplifying parts of the small ribosome subunit using primers for the conserved regions. We used two sets of primes – 27F (AGAGTTTGATCMTGGCTCAG) + 907R (CCGTCAATTCCTTTRAGTTT) and PEUF (GCAAACAGGATTAGATACCC) + 1195R (GACGTCRTCCNCDCCTTCCTC). The first set (27F + 907R) covers variable regions 1–4, and the second set (PEUF + 1195R) covers variable regions 4 (partly) – 7. The initial PCR reaction gave a product only when using the PEU + 1195 primer set, which we found to be more sensitive, as the PCR product is shorter. Nevertheless, the product was clear, and sequencing with both forward and reverse primers gave a 450bp of good, reliable sequence. Blasting the sequence to NCBI database resulted in *N. meningitidis* with 99% identity.

For convenience, the table below describes the chronical order of the tests, and their results.

chronical order	Test	Result
1	CSF culture	Negative
2	Blood culture	Negative
3	Enterovirus (PCR)	Not detected
4	BioFire ME/EC	*meningitides - positive* CMV – positive
5	CMV viral load	Not detected
6	16S sequencing	*Neisseria meningitidis. positive*

The infant was treated with IV antimicrobials for 7 days with a rapid clinical response and improvement of the abnormal leukocyte count and CRP. He was discharged after 8 days with a normal physical examination and no abnormal neurological sequela. Due to lack of specimen, CSF studies were not repeated

Discussion

We present a child with clinically- and laboratory-confirmed meningococcal meningitis. The clinical findings as well as the laboratory findings – leukocytosis with neutrophilia, elevated serum CRP, and a high leukocyte count in the CSF and the molecular CSF studies – are all indicative of this infection. In addition, the rapid clinical and laboratory responses to antimicrobials therapy support this diagnosis.

The role of the positive CMV in the BioFire FilmArray Meningitis/Encephalitis test is unclear. Taken the positive serum CMV serology, which indicates past infection (or maternal antibody) and the confirmed latency of CMV in blood white cells, the positive CSF CMV by the BioFire FilmArray Meningitis/Encephalitis assay is probably of no clinical significance. Although the company declare specificity of 99.8% (95% CI), false positive for CMV detection in the filmarray meningitis/encephalitis test has been reported [9]. On the other hand, the two different assays are probably using different primers, therefore a mutation can play a role in not detecting the virus. Since we did not have additional CSF specimen, we could not further investigate this issue. Therefore, we cannot determine if this is a case of false positive assay or a mild reactivation that was not detected. The use of filmarray meningitis/encephalitis test is becoming relatively common, as it provides a rapid and sensitive test. There is no doubt that the technique is a game changer, but the high sensitivity can be a double edged sword in the case of the herpesviruses. The latent nature of the Herpes viruses needs to be taken into account, and should be discussed whether this is the primary pathogen or a secondary reactivation. It is up to the physician and the laboratory to communicate and decide how to distinguish these cases. Physicians should be aware of this limitation of the very sensitive BioFire FilmArray Meningitis/Encephalitis molecular assay.

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Author's statement

Nadav Sorek – Conceptualization, Methodology, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Supervision, Project Administration.

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Conflicts of interest

The authors report no relevant conflicts of interest.

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