Evaluation of the Utilization of FilmArray Meningitis/Encephalitis in Children With Suspected Central Nervous System Infection
A Retrospective Case Series

Renan Barros Domingues, MD, PhD, Maria Cássia Mendes-Correa, MD, PhD,† Fernando Brunale Vilela de Moura Leite, MD, PhD,* Márcio Vega dos Santos, MD,* and Carlos Augusto Senne Soares, MD**

Background: The etiology of central nervous system infections is often difficult to establish. FilmArray meningitis/encephalitis (ME) panel is a multiplex polymerase chain reaction for rapid identification of 14 pathogens. The aim of this study was to evaluate potential real-life contributions of the use of this method in the pediatric population.

Methods: We herein report the results obtained with FilmArray ME in a retrospective case series of 367 children with suspected central nervous system infection. We identified viral and bacterial agents by FilmArray, and we evaluated the potential diagnostic contributions of the use of the panel taking into account the cytological, biochemical, and microbiological results of the cerebrospinal fluid (CSF) analysis.

Results: The FilmArray ME panel detected a viral infection in 186 cases (50.7%) and a bacterial infection in 12 cases (3.3%). Fifty-three cases (28.4%) of viral infection had at least 1 CSF finding that could be mistaken for bacterial meningitis. Enterovirus was identified in 2 cases with normal CSF findings. Among 12 bacterial infection cases, only 6 (50%) had a positive result with conventional microbiology analysis (Gram stain and culture). The CSF findings suggestive of bacterial meningitis were found in all 6 cases in which FilmArray was the only method to identify bacterial etiological agent.

Conclusions: FilmArray ME panel identified an etiological agent in cases in which conventional CSF analysis failed, providing potential clinical contributions to the management of such cases.

Key Words: meningitis, encephalitis, FilmArray, cerebrospinal fluid, enterovirus

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Central nervous system (CNS) infections are among the most severe infectious conditions as they are life-threatening and potentially associated with neurological sequelae.[1–3] The rapid diagnosis is essential for the early introduction of anti-infective treatment. However, the etiological definition is not easy given the wide variety of agents associated with these diseases, including viruses, bacteria, and fungi.[4,5] Cerebrospinal fluid (CSF) cytological and biochemical findings, as well as conventional microbiological methods, including staining methods, antigen detection, and culture, are of great help; however, they are not sensitive enough and may take a long time for a final result.[6–8]

FilmArray meningitis/encephalitis (ME) panel is a multiplex polymerase chain reaction (PCR) for rapid identification of 14 pathogens from CSF: *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Escherichia coli K1*, *Haemophylus influenza*, *Listeria monocytogenes*, cytomegalovirus (CMV), enterovirus, herpes simplex 1 and 2, human herpesvirus 6 (HHV-6), human parechovirus, varicella-zoster virus, and *Cryptococcus neoformans/gattii.*[9] It is being used increasingly worldwide, and previous studies suggest that FilmArray ME has high diagnostic accuracy.[10] Besides, it was shown that FilmArray ME panel reduces the time to microbiological result, allow earlier discontinuation of empirical anti-infective agents, and earlier hospital discharge.[11–13]

In the present study, we describe the results obtained with the use of FilmArray ME panel in cases in pediatric patients with suspected CNS infection and discuss the potential clinical impact of its utilization.

METHODS

This was a retrospective case series study in which we describe the results obtained with FilmArray ME among children with suspected CNS infection and to describe potential real-life clinical contributions that the use of FilmArray may have brought. We included all cases in which the pediatrician ordered FilmArray ME, from March 2019 to December 2019. The CSF samples were collected and analyzed by Senne Liquor Diagnóstico. The clinical assessment that led to suspicion of CNS infection and the CSF analyzes and FilmArray requests were made by pediatricians working in emergency units without the participation of Senne Liquor Diagnóstico staff members. The authors did not have access to the clinical data of the patients. As soon as the pediatrician orders CSF, our laboratory is called and immediately goes to the hospital to proceed with the collection and subsequent CSF analysis. After analysis, the CSF results are stored in the Tasy platform (Philips Healthcare). This workflow is routinely adopted by our laboratory and hospitals that we provide CSF services and has not been changed because of the present study. A database was created in which all the FilmArray cases performed during the mentioned period were included. This database included CSF data, age, and sex of the patients. The patients were assisted in 19 different private hospitals in São Paulo and Campinas in which Senne Liquor Diagnóstico in which Senne Liquor Diagnóstico is in charge of CSF collections and analyses. All authors of the present study have no relationship with the FilmArray manufacturer. The doctors requesting CSF collection and analyses, including FilmArray, have no relationship with the authors of the article. Senne Liquor Diagnóstico is the only service to carry out CSF collections and analyses in these hospitals. The data presented in this article were extracted from a study approved by the research ethics committee

From the *Senne Liquor Diagnóstico; and †Virology Section (LIM-52), Instituto de Medicina Tropical, Universidade de São Paulo, São Paulo, Brazil.
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Reprints: Renan Barros Domingues, MD, PhD, Senne Liquor Diagnóstico, Avenida Angélica, 2071, 3o Andar, CEP 01227-200, São Paulo, SP, Brazil (e-mail: renan.domingues@senneliquor.com.br).

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FilmArray was carried out according to the manufacturer's instructions. Basically, 400 μL of CSF was subject to FilmArray ME panel testing that consists of automated nucleic acid extraction, reverse transcription, nucleic acid amplification, and result analysis. For data analysis, the FilmArray ME results were categorized into negative, viral meningitis when a virus was identified and bacterial meningitis when the panel was positive for bacteria. The CSF data included in the present analysis were obtained from the Senne Liquor Diagnóstico database and included white blood cell (WBC) count (in cubic millimeters), neutrophil (in percentages), protein (in milligrams per deciliter), glucose (in milligrams per deciliter), and lactate concentration (in milligrams per deciliter). The adopted reference values for normal CSF findings were as follows: WBC, ≤3 cells/mm^3 (newborn ≤30 leukocytes/mm^3; 28 days to 3 months ≤9 leukocytes/mm^3); protein, ≤40 mg/dL (premature infants <150 mg/dL and full-term newborns <100 mg/dL); glucose, ≥40 mg/dL or ≥2/3 serum glucose (premature infants >20 mg/dL and full-term newborns >30 mg/dL); and lactate, ≤20 mg/dL. The CSF findings that were considered suggestive of bacterial meningitis were as follows: >1000 WBC/mm^3; neutrophils, >80%; glucose, <40 mg/dL; protein, >200 mg/dL; or lactate, >30 mg/dL.[13]

RESULTS

Three hundred sixty-seven CSF samples successfully obtained from 367 children with suspected CNS infection were submitted to FilmArray ME panel analysis. The age ranged from 0 to 14 years. The mean age was 4.04 ± 3.39 years. There were 211 male and 156 female patients. The CSF WBC ranged from 0 to 8620/mm^3 (mean ± SD, 296.65 ± 696.71 WBC/mm^3). The CSF protein ranged from 10 to 862 mg/dL (mean ± SD, 54.68 ± 75.32 mg/dL). The CSF glucose ranged from 5 to 156 mg/dL (mean ± SD, 53.68 ± 75.32 mg/dL). The CSF lactate ranged from 10 to 117.2 mg/dL (mean ± SD, 18.86 ± 11.66 mg/dL). Thirty-seven patients had all normal CSF findings (WBC, protein, glucose, and lactate). Fifty-five CSF samples (14.9%) were bloody, and 28 (50.9%) of them were positive with FilmArray. The percentage of FilmArray positive cases in nontraumatic CSF samples (55.4%) was not significantly different from traumatic CSF samples ($P = 0.53$).

The FilmArray ME panel results were negative in 169 CSF samples (46%), positive with identification of a bacterial agent (bacterial infection) in 12 (3.3%), and positive with identification of virus (viral infection) in 186 (50.7%). Demographic and laboratorial data of these 3 groups are shown in Table 1. The results in viral and bacterial cases will be reported separately.

Viral Infection

FilmArray was positive for virus in 186 cases (Table 1). Enterovirus was the most frequent agent identified by FilmArray ME panel, being identified in 182 (97.85%) of the 186 viral infection cases and in 91.9% of all positive cases. Enterovirus was identified as the only agent in 169 cases and in association with HHV-6 in 13 cases. Other viral infections included HHV-6 that was the only identified agent in 3 cases and CMV that was identified in 1 case.

In 53 cases (28.4%) of viral infection, there was at least 1 CSF finding suggestive of bacterial meningitis. The CSF with greater than 1000 WBC/mm^3 was found in 15 (8.06%) of the 186 viral meningitis cases. Twenty-five viral meningitis cases (13.44%) had more than 80% polymorphonuclear cells. The CSF biochemical findings suggestive of bacterial meningitis were also found in viral infection cases: glucose, <40 mg/dL (26 cases, 13.98%); and lactate, >30 mg/dL (2 cases, 1.07%). A protein level of greater than 200 mg/dL was not found among the viral meningitis cases. In all but 1 case, the viral infection with CSF data was suggestive of bacterial meningitis, and the identified agent was enterovirus. In 1 case, the identified agent was CMV. Enterovirus was identified in 2 cases with completely normal CSF findings.

HHV-6 was identified in association with other agents. In 13 cases, it was identified in association with enterovirus and in 1 case with *Neisseria meningitidis*. In 3 cases, it was the only identified agent. In 2 of these cases, there were CSF inflammatory parameters with mild pleocytosis (47 and 30 WBC/mm^3) with normal glucose and lactate concentrations. In 1 case, there was a marked lymphocytic pleocytosis (1125 WBC/mm^3) with a slight increase in protein and lactate levels. Four HHV-6–positive cases were bloody CSF samples.

Bacterial Meningitis

FilmArray ME panel identified bacterial infection in 12 patients: *N. meningitidis* (7 cases); *H. influenza* (4 cases); and *S. pneumoniae* (1 case). The CSF findings in this group were as follows: 440 ± 2608.3 WBC/mm^3; CSF protein, 162 ± 72.6 mg/dL; CSF glucose, 21.2 ± 20.4 mg/dL; and CSF lactate, 65.4 ± 27.6 mg/dL. None of these cases were already on anti-infective agents when CSF was collected.

Only 6 of these cases had positive results with conventional microbiological methods (Gram stain and culture; *N. meningitidis*, 4 cases; *H. influenza*, 2 cases). Both culture and Gram stain were positive in 1 case, culture was positive alone 3 cases, and Gram stain was positive alone in 2 cases. In all the CSF culture and CSF bacterioscopy positive cases, conventional microbiology and FilmArray ME panel identified the same agent.

In 6 patients, FilmArray ME panel was the only method to identify the bacterial etiological agent. In 3 patients, the identified agent was *N. meningitidis*; in 2, the identified agent was *H. influenza;* and in 1, the identified agent was *S. pneumonia*. All these cases had inflammatory CSF findings that are shown in Table 2.

DISCUSSION

The described results indicate that FilmArray ME panel can potentially contribute in the real-life clinical evaluation of children with suspected CNS infection. Nearly 28% of the children with positive FilmArray for viral infection had CSF findings

TABLE 1. Demographic and Laboratorial Information

FilmArray Result	Age, Mean ± SD, y	Male/ Female	WBC, mm^3	Protein, mg/dL	Glucose, mg/dL	Lactate, mg/dL
Negative (n = 146, 46%)	3.81 ± 3.68	1.22	165.71 ± 34.13	62.41 ± 101.74	55.93 ± 18.11	16.47 ± 7.91
Viral infection (n = 185, 57%)	4.31 ± 3.01	1.55	287.18 ± 411.13	40.75 ± 23.07	53.34 ± 12.33	18.03 ± 4.47
Bacterial infection (n = 12, 3.3%)	3.08 ± 3.17	3	2276.50 ± 2608.27	162.5 ± 72.59	21.17 ± 20.46	65.43 ± 27.63

TABLE 2. Cerebrospinal Fluid Findings and FilmArray ME Panel Results in Patients With Bacterial Meningitis With Negative Results in Conventional Microbiology Tests

Age	Sex	WBC/mm^3	Neutrophils, %	Protein, mg/dL	Glucose, mg/dL	Lactate, mg/dL	FilmArray ME
5	Male	30	16	24	61	15.3	*Neisseria meningitidis*
2	Female	410	38	95	23	24.6	*Neisseria meningitidis*
4	Female	970	70	140	27	63.7	*Streptococcus pneumoniae*
1	Female	1088	65	205	38	45.9	*Haemophylus influenza*
1	Female	2960	90	99	5	68.5	*Haemophylus influenza*
11	Male	6160	75	166	56	70.4	*Neisseria meningitidis*

suggestive of bacterial meningitis. In such cases, FilmArray can potentially contribute to the clinical decision to stopping anti-infective agents in cases where, although CSF is suggestive of bacterial meningitis, PCR is positive for virus and negative for bacteria, and cultures are negative. This finding is in line with previous studies showing that the use of FilmArray ME panel lead to earlier discontinuation of empirical antibiotics and earlier hospital discharge in cases with negative cultures and negative PCR for bacteria.[11] These outcomes may be associated with cost reduction due to shorter hospitalization and with lower risks resulting from unnecessary medication use.[14,15]

Another potential contribution was the identification of a viral etiologic agent in cases of normal CSF findings. A prior study already showed that restricting FilmArray ME panel orders based on pleocytosis or other CSF abnormalities can result in missed diagnosis opportunities.[16] In that study, nearly 20% of patients with no pleocytosis and normal CSF glucose and protein had positive result with FilmArray ME panel. The identification of an etiological agent brings relevant information to the physician, the patient, and the family and can contribute to avoid further unnecessary diagnostic tests and clarifying the cause of the symptoms. This can potentially contribute to reduce the length of hospital stay in these cases. It could be argued that the use of PCR for enterovirus would be a cheaper alternative than the use of the panel. The costs are approximately 3 times lower with single-agent PCR. However, the disadvantage is that enterovirus PCR alone does not identify other possible viral etiologic agents. In addition, the demonstration of a negative result for bacteria increases the probability of viral etiology, particularly in cases in which general CSF analysis may suggest bacterial infection.

FilmArray ME panel brought potential clinical contribution in cases with bacterial infection. In 50% of the bacterial infection cases, FilmArray ME panel was the only method to identify the etiologic agent. It could be argued that these were false-positive results with FilmArray ME panel; however, the presence of CSF abnormalities suggestive of bacterial infection speaks against this possibility. In addition, the existing literature shows high accuracy of FilmArray for the diagnosis of bacterial meningitis.[10] In a previous meta-analysis, the false-positive rate was 4% after clinical adjudication of the results with the highest proportion observed for *S. pneumoniae* and *S. agalactiae.* In our study, only 1 patient was diagnosed by FilmArray with *S. pneumoniae* meningitis with negative conventional microbiology and no cases of meningitis by *S. agalactiae*. The patient with *S. pneumoniae* meningitis had CSF with 970 WBC/mm^3, with 70% of neutrophils, a protein level of 140 mg/dL, a glucose level of 27 mg/dL, and a lactate level of 63.7 mg/dL. This CSF pattern clearly indicates a very high probability of bacterial meningitis, and conventional microbiology is more likely to have provided false-negative result than that FilmArray a false-positive result. This finding is in line with previous studies that already showed the ability of FilmArray ME to increase the diagnostic sensitivity in cases of bacterial meningitis, being sometimes the only positive test for the identification of an etiological agent.[17–19]

Besides the diagnostic advantages presented previously, a great advantage is the short time of the result that can be obtained in up to 1 1/2 hours. However, there are some concerns and interpretation difficulties with FilmArray ME panel. One relates to HHV-6 results. Human herpesvirus 6 was often detected in association with other agents. Previous studies showed that HHV-6 can be identified during CNS infections by FilmArray ME panel in cases in which this finding is unlikely to be clinically significant.[20] This may occur because nucleic acids of HHV-6 can also be detected in latency and asymptomatic viral reactivation in association with blood-brain-barrier dysfunction during a CNS infection due to other etiologic agent. Therefore, the HHV-6 results must be interpreted with caution, and therapeutic decisions cannot be taken based solely on the result of FilmArray ME panel.[21] Another concern regarding HHV-6 is that this virus infects peripheral blood cells and therefore may contaminate CSF in traumatic tap. Apparently, this was not relevant in our sample because only 4 of 13 HHV-6–positive cases were bloody CSF. Another disadvantage of FilmArray is that the panel does not include important CNS infection etiologic agents, such as Epstein-Barr and *Mycobacterium tuberculosis*. Other disadvantage is the inclusion of etiological agents, such as human parechovirus, that does not seem to be relevant in many regions, such as our country, thus contributing only to the cost increase of the method.[22] The high cost is certainly an important limiting factor for a more widespread use of the panel.

The present study has limitations. First, it was a retrospective study with access only to the CSF database, without clinical and radiological correlations. We only assessed samples from private hospitals, so our sample is not representative of the Brazilian population. Considering that this was a retrospective study including only cases in which FilmArray was ordered, it was not possible to access sensitivity and specificity of FilmArray. In addition, we could not evaluate cost-effectiveness of the use of FilmArray, not allowing us to fully evaluate the impact of utilization of FilmArray ME panel in the clinical routine. We could not exclude false-positive results in cases in which FilmArray was the only positive test. However, the large number of cases strengthens our findings and indicates a potential clinical contribution of FilmArray in cases where it is the only positive method for the identification of the etiological agent.

In conclusion, we showed that FilmArray ME panel may be helpful for pediatricians in their clinical decisions when assessing cases of suspected CNS infection in emergency units.[23–25] This help can be by identifying an etiological agent in cases with unexpected CSF findings, detection of an etiological agent when conventional microbiology is negative, and allowing a faster identification of the causative agent. A more widespread use of this panel is desirable. Further studies will more clearly identify the economic advantages of performing this panel in all cases of acute CNS infections.

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