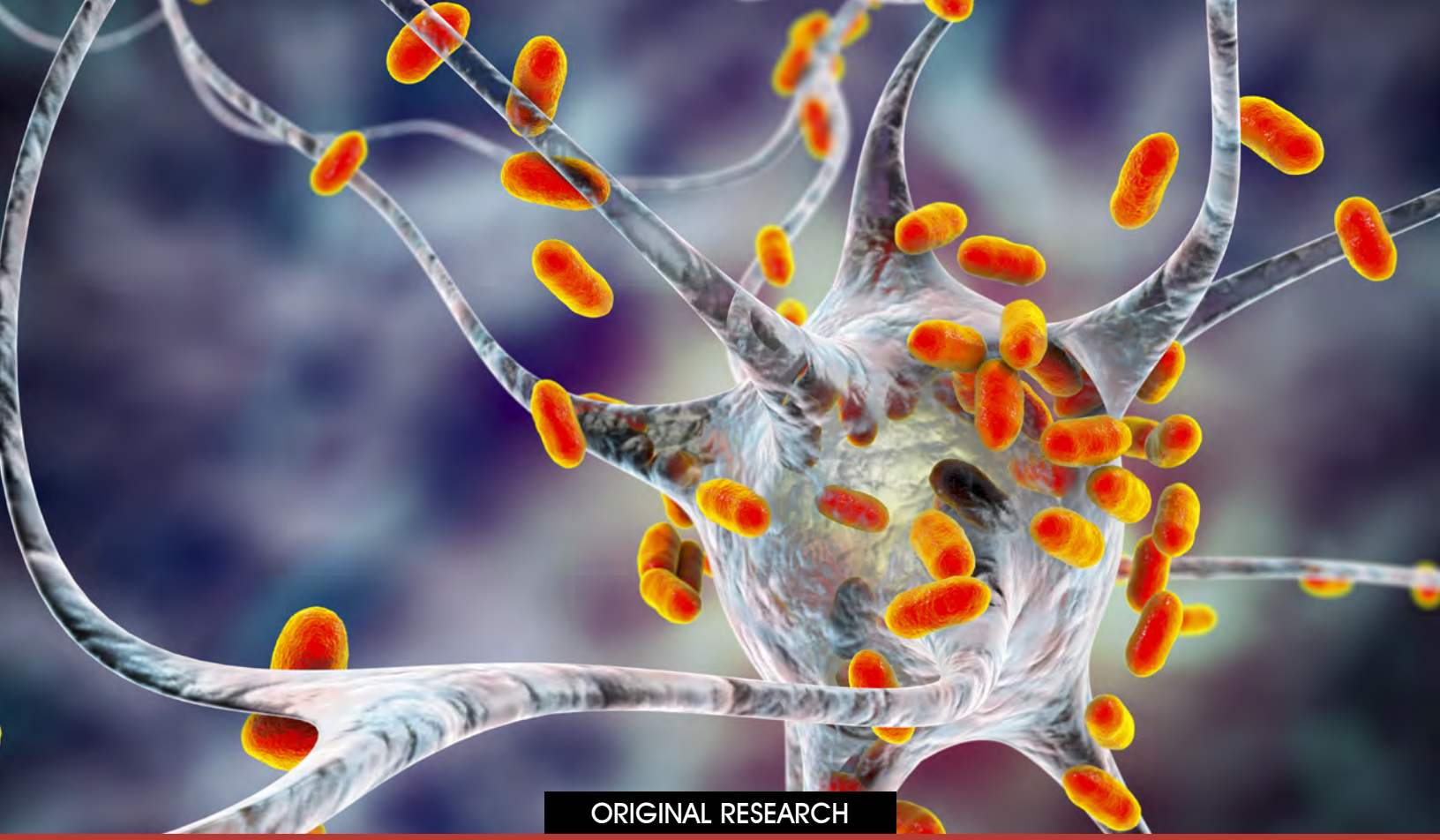

ORIGINAL RESEARCH

Correlation of Meningitis/Encephalitis PCR Panel Results with CSF Pleocytosis

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ABSTRACT

Background

This study investigates the correlation between CSF pleocytosis and the results of a Meningitis/Encephalitis PCR Panel (ME panel). We hypothesize that CSF pleocytosis is not a predictive marker of disease in aseptic CNS infections.

Methods

A retrospective chart review was completed of 147 patients who underwent lumbar puncture over 1 year at a large, tertiary pediatric hospital. Ages, CSF cell counts, and ME panel results were obtained. The clinical usefulness of using pleocytosis (WBC>5cells/mcL) as a predictive marker for CNS infections was analyzed.

Results

Of the 147 patients included, 99 patients (67%) were noted to have negative pleocytosis, while 48 patients (33%) were noted to have pleocytosis. Of the 48 with pleocytosis, 18 (38%) had a positive ME panel, while 30 (62%) had a negative ME panel. In the 99 patients with negative pleocytosis, 9 (9%) had positive ME panel, and 90 (91%) had negative ME panel. The sensitivity and specificity of CSF pleocytosis as a marker for disease was 67% and 75%, respectively, with a positive predictive value of 38% and a negative predictive value of 91%. In patients with a positive ME panel with a confirmed viral source, there was a statistically significant lower percentage of CSF pleocytosis in infants <12 months of age compared with older children (p =.002).

Conclusions

This chart review demonstrates that CSF pleocytosis is not a good indicator of CNS infection, especially in cases due to viral etiologies. This is important in younger infants who may not be able to mount an appropriate inflammatory response against viral pathogens in CSF. The ME panel can quickly identify viral pathogens thereby decreasing antibiotic treatment and hospital stays. We recommend the routine use of ME panels, particularly in young infants, regardless of initial CSF cell counts.

INTRODUCTION

Aseptic meningitis is a common pediatric diagnosis, classically defined as acute onset of meningeal signs or symptoms with fever and cerebrospinal fluid (CSF) pleocytosis in the absence of a positive bacterial culture and gram stain.[1] Because of this traditional definition of meningitis, it has largely remained a diagnosis associated with pleocytosis, defined as CSF WBC > 5 cells/mcL. The most common etiology of aseptic meningitis is usually viral, and although usually self-limited and self-resolving, may sometimes be associated with significant morbidity, particularly in infants less than one year of age. Infants may present with excessive obtundation, signs of increased intracranial pressure, focal seizures, and other focal neurological sequelae.[2] CSF Gram stain and culture have been the diagnostic "gold standard" for acute meningitis for nearly a century. However, both methods have a limited sensitivity that may be further reduced in patients who have received antibiotics or in patients with a viral source. Molecular assays have the potential to have a more rapid turnaround time, are potentially less affected by prior antimicrobial therapy, and have higher diagnostic yield.[3] A FilmArray Meningitis/Encephalitis (ME) panel has recently been developed which rapidly tests for 14 common organisms (bacterial, viral, and fungal) causing central nervous system (CNS) infections via multiplex polymerase chain reaction (PCR).[4] Because of the increased sensitivity of PCR testing and rapid availability of results, it is possible to identify many organisms responsible for aseptic meningitis that heretofore remained undefined, which in turn may affect length of stay, judicious treatment choices, and duration of therapy[5] With such a sensitive molecular test available to identify CNS infection, it is possible to diagnose meningitis, which in the absence of pleocytosis, may cause significant morbidity in this vulnerable population. The hypothesis of this study is that CSF WBC count *alone* is an unreliable marker of infection. Hence, the purpose of this retrospective study is to investigate the correlation between CSF cell counts and the results of a recently implemented ME panel at a large tertiary pediatric hospital.

METHODS

We conducted a retrospective study at a 289-bed, freestanding, children's hospital in the Southeastern US from May 2016 to March 2017. Initially, all children who underwent lumbar puncture with a concurrent FilmArray ME panel were reviewed. Study inclusion criteria included any patient who had a lumbar puncture performed with CSF cell counts and culture in addition to a FilmArray ME panel with available results. The population evaluated included pediatric patients, ages 1 day to 18 years of age. There were no predefined restrictions in the study based on gender, age, or race. Initial review included 189 patients that met inclusion criteria, however those with resultant traumatic lumbar punctures (as defined by CSF red blood cell count >500 cells/mcL) were excluded, leaving 147 charts to be reviewed. Patients' ages, CSF cell counts, and FilmArray ME panel PCR results were obtained. Statistical analysis, including a Fisher Exact Test, were performed to analyze the clinical usefulness of using pleocytosis (WBC >5 cells/mcL) as a predictive marker for CNS infections and the subsequent utility of the FilmArray ME panel. This chart review was approved by the institutional review board at the participating children's hospital.

RESULTS

Of the 147 patients included in the study, 99 (67%) were noted to have negative pleocytosis, while 48 (33%) were noted to have pleocytosis (Table 1). Of those 48 patients with pleocytosis, 18 (38%) had a positive ME panel, and 30 (62%) had a negative ME panel. In the 99 patients with negative pleocytosis, 9 (9%) had a positive ME panel, while 90 (91%) had a negative ME panel. In the patients with a positive ME panel without pleocytosis (n=9), 8 (89%) were due to viral etiologies, the majority of which were in patients <12 months of age (6/8, [75%]), and 1 patient had a positive ME panel for *Escherichia coli* with negative pleocytosis. Of the viral cases without pleocytosis (n=8), 4 (50%) cases were secondary to parechovirus, 2 (25%) to enterovirus, and 1 (13%) each to HHV-6 and HSV-1 (Table 2).

In the group with pleocytosis and a positive ME panel (n=18), 14 patients (75%) demonstrated viral etiologies detected on the ME panel. Only 1 (7%) of these 14 viral cases occurred in a patient <12 months of age. There were 13 (93%) cases of enterovirus and 1 (7%) case of HHV-6.

When comparing infants younger than 12 months of age with viral meningitis/encephalitis diagnosed by the ME panel with their older counterparts, there is a statistically significant lower percentage of CSF pleocytosis in infants as a marker for

RESULTS ME PANEL	POSITIVE PLEOCYTOSIS	NEGATIVE PLEOCYTOSIS	TOTAL
POSITIVE	12% (18/147)	6% (9/147)	27
NEGATIVE	20% (30/147)	61% (90/147)	120
TOTAL	48	99	

Table 1: Prescence of pleocytosis according to Film Array Meningitis Encephalitis Panel

RESULT	ME PANEL POSITIVE (NO. PATIENTS)	POSITIVE PLEOCYTOSIS	NEGATIVE PLEOCYTOSIS
POSITIVE	27	18	9
Haemophilus influenzae	2	2	0
Escherichia coli	1	0	1
Streptococcus pneumoniae	1	1	0
Parechovirus	4	0	4
Enterovirus	15	13	2
Human herpesvirus 6	2	1	1
Herpes simplex virus 1	1	0	1
Cryptoccocus neoformans	1	1	0
NEGATIVE	120	30	90

Table 2: Association of organism-specific PCR results with pleocytosis

disease, (Fisher Exact Test; p=.002). The overall sensitivity and specificity of CSF pleocytosis as a marker for disease was 67% and 75%, respectively, with a positive predictive value of 38% and a negative predictive value of 91%.

DISCUSSION

The initial purpose of this study was to investigate the correlation between the presence of CSF pleocytosis and the results of a FilmArray Meningitis/Encephalitis PCR Panel at a large tertiary pediatric hospital. This chart review demonstrates that presence or absence of CSF pleocytosis is not a good indicator of CNS infection, especially in cases due to viral etiologies. Our findings indicate that the ME panel has a high negative predictive value (91%), which supports the likelihood that a negative ME panel is an accurate predictor of truly negative results. Additionally, in all the patients that had a negative ME panel, regardless of the presence of pleocytosis, all corresponding CSF cultures and gram stains were also negative. In cases of viral etiologies in infants <12 months of age, there was a statistically significant difference (p=.002) in the presence of CSF pleocytosis. Younger infants were less likely to have pleocytosis despite demonstrating positive viral sources in the ME panel. This is a particularly important distinction for clinicians, since due to age and possible lack of immune maturity, infants may not be able to mount an appropriate inflammatory response in the CSF against viral pathogens.[6] Due to the absence of pleocytosis, the diagnosis of aseptic meningitis, in this age group may be delayed or missed, hence highlighting the diagnostic utility of a PCR test.

In a study by Precit et al[7], they investigated if the presence of certain clinical parameters, including CSF pleocytosis provided diagnostic accuracy for positive ME panel results in pediatric patients. They restricted ME panel testing to patients with abnormal CSF findings (pleocytosis, abnormal protein, and glucose levels in the CSF). Among positive ME panel specimens, sensitivity and positive predictive values were <90% for all biomarkers. CSF pleocytosis and abnormal glucose/protein were poor predictors of ME panel positivity, and they concluded that ME panels should not be restricted to patients with abnormal CSF parameters.

In a prospective cohort study conducted by Posnakoglou et al[8], children with suspected CNS infection and CSF pleocytosis were randomized 1:1 to a group undergoing ME panel testing or to a cohort group conducting separate molecular CSF microbiological tests. A total of 71 cases were included, and a pathogen was detected in 37(52.1%) of children when the

ME panel was used and in 16(22.5%) in the control group ($p < .001$). In aseptic meningitis cases, a virus was detected in 27/61(44.2%) patients with ME and in 11/66(16.7%) of controls ($p<.001$). These findings reflect that the ME panel detected significantly more CNS pathogens, both bacterial and viral, than in the cohort group.

We identified several limitations in our study. First, the chart review included patients from a single center, thus introducing the possibility that the patient population had inherent differences, whether culturally or geographically, from the entire population, and therefore was not wholly representative or reflective. Second, at the time of the chart review, the ME panel was a fairly new test, having only been released one year prior. Given how recent the test was at the time, we could not conclude if the test was always ordered despite signs of CSF pleocytosis on initial CSF studies and/or signs of clinical meningitis/encephalitis. This fact may have affected the conclusions' extension from the study conducted on a sample population to a larger population. Third, in one of the patients with a positive ME panel with negative pleocytosis (n=9), a bacterial etiology, *E.coli*, was demonstrated in the PCR. Upon chart review, this patient was a 2-week-old male infant who presented with low-grade fever and URI symptoms, secondary to RSV infection. During admission, his urine culture grew 2,000 CFU/mL of *E.coli*, after which, due to his age, the decision was made to perform lumbar puncture. CSF studies were within normal ranges, including negative CSF culture and Gram stain, but ME panel was positive for *E.coli*. Given the well-appearance of the patient, the confirmed respiratory viral source of symptoms, the insufficient growth on urine culture of the pathogen, and negative CSF culture and Gram stain, we believe that there exists the possibility that the CSF PCR result was a possible contaminant.

Aseptic meningitis refers to meningeal inflammation in which a bacterial source is not identified. Of the recognizable viral pathogens, we note that non-polio enteroviruses are the leading cause of aseptic meningitis in the population studied. Although not studied in this chart review, it is accepted that prognosis, in terms of morbidity, largely depends on the age of the child, infectious agent, and immune status. While the majority of cases of pediatric viral meningitis can expect a complete recovery, some infants and children younger than 2 years of age have been shown to experience acute neurological sequelae as a result of infection, such as complex seizures, increased intracranial pressure, coma, or long-term behavioral changes.[2] While CSF culture and gram stain remain the gold standard for diagnostic purposes, the rapid detection of an etiologic agent via the FilmArray ME panel, not only aids in timely diagnosis, but has the potential to decrease adverse outcomes, prolonged hospitalizations, and unnecessary antibiotic treatment. While further studies are needed to determine the cost effectiveness and clinical parameters of this new test, we suggest the routine use of ME panels, particularly in young infants, regardless of initial CSF cell counts, in conjunction with traditional diagnostic CSF studies.

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