INTRODUCTION

Meningitis constitutes a significant factor in the high mortality and morbidity rates worldwide. Each year, an estimated 500,000 cases of meningitis occur globally, with bacterial cases posing a potential life-threatening risk, resulting in a mortality rate of 1 in 10 patients and serious complications in 1 in 5 patients. In 2019, the World Health Organization (WHO) reported approximately 250,000 deaths due to meningitis. In Indonesia, the Ministry of Health recorded 19,381 cases of meningitis in 2010, with a death toll of 1,025 individuals. Consequently, establishing the etiological diagnosis of meningitis becomes a crucial concern, and cerebrospinal fluid (CSF) analysis emerges as pivotal in this endeavor.

Meningitis is an inflammatory disease that affects the tissues guarding the brain and spinal cord, and alterations in cerebrospinal fluid (CSF) can indicate the presence of infection. Additional distinguishing features of meningitis from other benign childhood illnesses include leg pain, cold extremities, and abnormal skin coloration. This cerebrospinal fluid plays a vital role in maintaining chemical balance in the central nervous system and safeguarding the brain against mechanical trauma. The analysis of cerebrospinal fluid constitutes a key element in the diagnosis and management of this disease. The most significant examination to identify or rule out the presence of meningitis is the analysis of cerebrospinal fluid via lumbar puncture (LP). Nonetheless, the interpretation of cerebrospinal fluid examination results necessitates caution, particularly due to changes in CSF characteristics such as leukocyte count, protein levels, glucose, and physical appearance, which can provide insights into the type of disease occurring. CSF analysis is crucial for diagnosis since clinical characteristics alone cannot differentiate between meningitis and similar diseases, and the analysis of CSF in bacterial meningitis presents distinct characteristics compared to non-bacterial meningitis.

In the effort to swiftly identify the cause of meningitis infection, various diagnostic methods have been developed. Apart from standard procedures like Gram staining and culturing cerebrospinal fluid, Polymerase Chain Reaction (PCR) technology has been employed to detect pathogens in cerebrospinal fluid. However, culture methods have limitations in terms of time and sensitivity. The Biofire FilmArray ME panel employs multiplex PCR technology to detect various pathogens causing meningitis in approximately 1 hour. This
panel can rapidly and accurately identify various bacteria, viruses, and fungi causing meningitis. The prompt diagnosis of the etiology of meningitis is essential for timely and efficient treatment.17 This study aims to compare the results of cerebrospinal fluid analysis with the Biofire Filmarray ME panel in an effort to expedite and facilitate the establishment of the etiological diagnosis of meningitis.

METHODS

This research was implemented using an analytical observational design with a cross-sectional approach on a population of meningitis patients.

Sample Collection

This study was conducted over a 4-month period, from February 2023 to May 2023. The research sites included several hospitals, namely the Clinical Pathology Installation of dr. Soetomo Hospital for CSF sample collection, CSF analysis, and CSF analysis with multiplex PCR. Additionally, CSF sampling was conducted at dr. Soetomo Hospital, Adi Husada Hospital, Mitra Keluarga Hospital Waru, Mitra Keluarga Hospital Satelit, Soewandhi Hospital, Al Irsyad Hospital, and Siloam Hospital Surabaya.

The study population consisted of patients diagnosed with meningitis by neurology specialists or neurology consultant pediatric specialists, both in outpatient and inpatient settings at the research locations. The variables observed in this study were the results of the Biofire Filmarray ME panel examination and the analysis of cerebrospinal fluid (CSF) results.

Research Procedure

The laboratory procedures involved in the analysis of the Biofire Filmarray ME Panel and cerebrospinal fluid (CSF) analysis encompassed several systematic stages.

First, the examination principle of the Biofire Filmarray ME Panel was based on multiplex PCR methodology. This method enables the simultaneous amplification of DNA template regions using multiple different primers in a single PCR reaction. The Biofire Filmarray ME Panel examination process began by extracting the Filmarray pouch from its vacuum-sealed packaging. Subsequently, the Filmarray ME pouch was placed in the Filmarray Pouch Loading Station, designed to prevent errors. The Hydration Solution was loaded into the Filmarray ME pouch using the Hydration Injection Vial. The Sample Buffer was drawn from the ampoule and added to the Sample Injection Vial along with the CSF specimen using a Transfer Pipette. After thorough mixing, the sample-buffer mixture was introduced into the Filmarray ME pouch using the Sample Injection Vial. Process controls were monitored and inserted into the pouch.

Furthermore, there were two main stages in the Biofire Filmarray ME Panel examination. The first stage involved nucleic acid purification, which occurred in the first three blisters of the pouch. The sample was activated, and the released nucleic acid was captured, washed, and eluted using magnetic bead technology. Reverse transcription was conducted to convert RNA viruses into cDNA before amplification. The purified nucleic acid solution was combined with a master mix and proceeded to PCR. The second stage involved diluting the products of the first-stage PCR, mixing them with PCR reagents containing intercalating fluorescent DNA dye, and distributing them through the second-stage PCR array.

Upon completion of the PCR process, DNA melting analysis was performed by gradually increasing the temperature and monitoring fluorescence in each well to generate a melting curve. The software could automatically interpret these analysis results.

On the other hand, CSF analysis on the Sysmex XN 1000 utilized flow cytometry. The examiner performed a background check procedure on this instrument by scanning the sample identification (SID) barcode before introducing the CSF sample into the machine.

In the Dimension EXL, CSF analysis involved several parameters such as glucose and total protein. The process commenced by scanning the SID barcode, inputting the desired analysis parameters, and introducing the sample tube into the instrument.

Subsequently, the data from the Biofire Filmarray ME Panel examination and CSF analysis were collected and recorded on the data collection sheet. Demographic data were descriptively presented through tables, figures, and written explanations for data presentation.

Data Analysis

Data analysis was conducted in several stages. Univariate analysis was performed using descriptive analysis to understand the characteristics of the investigated variables. Meanwhile, bivariate analysis was executed to ascertain the correlation between CSF analysis results and pathogens detected in the Biofire Filmarray instrument. Statistical analysis was carried out using the McNemar test.

RESULTS

Quality assurance of cerebrospinal fluid (CSF) examination represents the initial phase of this research. Prior to measuring the samples, checks were conducted for expiration dates, lot numbers of each kit, and proper reagent storage according to kit instructions. Quality control (QC) was performed daily to ensure the fulfillment of level 1 and level 2 ranges. The total protein measurement method employed UV spectrophotometry, wherein the red pyrogallol and sodium molybdate form a red complex with a maximum absorption at 470 nm. Proteins in the sample form a bluish-purple complex with the aforementioned complex in an acidic solution, absorbing at 600 nm. The results of this analysis were evaluated using logit curve fitting against a previous calibration curve.

The results of the cerebrospinal fluid (CSF) analysis indicated that out of 30 samples, 83.3% exhibited clear clarity, 93.3% showed no clotting, and 83.3% had a clear coloration. The cell count increased in 56.7% of the samples, protein levels increased in 43.3% of the samples, and glucose levels were normal in 56.7% of the samples. The conclusions drawn from the CSF analysis indicated that 70% leaned towards normal results, 3.3% indicated viral infection, and 26.7% pointed towards bacterial infection.

The results of the Biofire Filmarray ME panel examination demonstrated that 96.7% yielded negative results, with only 3.3% indicating positive results for a virus (Cytomegalovirus).
## Table 1. LCS Analysis Results

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<th>% PMN</th>
<th>% MN</th>
<th>Protein Total (mg/dL)</th>
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<td>56.4</td>
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</table>
Through graphs and tables, sample characteristics were analyzed. A comparison between the CSF analysis results and the Biofire Filmarray ME panel results was also conducted. The McNemar test revealed a significant difference between the CSF analysis results and the Biofire Filmarray ME panel results.

Overall, this research provides in-depth insights into CSF analysis and offers an understanding of the comparison between analysis results and the Biofire Filmarray ME panel method.

**DISCUSSION**

**CSF Analysis Results**

The CSF analysis results were categorized according to Ropper (2005) into 5 categories: normal, viral infection, bacterial infection, tubercular infection, and fungal infection. Out of 30 research samples, there were 9 (30%) samples with CSF analysis results indicative of normal findings (characterized by normal cell count, total protein, and glucose levels), 9 (30%) samples indicating bacterial infection (characterized by elevated cell count, increased total protein, and decreased glucose levels), 4 (13.3%) samples suggesting partially treated bacterial infection (noted by slightly increased cell count, elevated total protein, and reduced glucose levels), and 8 (26.7%) samples pointing towards viral infection (marked by slightly increased cell count, normal or increased total protein, but normal glucose levels). The interpretation of these CSF analysis conclusions was carried out independently by Neurology Specialists.

**Biofire Filmarray Examination Results**

Many of the Biofire Filmarray examination results were negative, aligning with other studies which reported relatively low positivity rates, such as 8.7% (136 positive out of 1424 samples), 12.6% (89 positive out of 734 samples), and 14.3% (6 positive out of 42 samples). Positivity rate refers to the percentage of positive results compared to the total examinations. This percentage can be employed to monitor and assess the severity and spread of a disease within a region or population. The higher the positivity rate for meningitis, the greater the likelihood of an outbreak and disease dissemination.

The low positivity rate in the Biofire Filmarray could be attributed to several factors. Firstly, inappropriate patient selection may yield tests with a low positivity rate. This occurs when tested patients lack symptoms or signs corresponding to the infections targeted by the Biofire Filmarray. Secondly, improper or less accurate testing techniques can lead to inaccurate test results and ultimately contribute to a low positivity rate. Thirdly, the limited sensitivity of the Biofire Filmarray test may result in false negative outcomes, ultimately leading to inaccurate positivity rate calculations. Lastly, a low disease prevalence in a specific region or population can also contribute to a low positivity rate. If the disease targeted by the Biofire Filmarray test is rare, then the positivity rate is likely to be low as well.

The results of CSF analysis can provide insights into the potential causes of meningitis/encephalitis, encompassing bacterial, viral, tuberculosis, fungal, or aseptic infections. In this study, there were 9 CSF analyses suggesting possible bacterial infections and 4 CSF analyses indicating possible partially treated bacterial infections, though their corresponding Biofire Filmarray results were negative. This could be attributed to various factors such as patients having received antibiotics prior to testing, presence of meningitis/encephalitis-causing microorganisms beyond the 14 pathogens detectable by the tool, or microorganism counts below the Limit of Detection (LoD) of the instrument.

The administration of antibiotics before CSF sample collection can work by killing bacteria (bactericidal) or inhibiting the growth and development of bacteria (bacteriostatic) causing meningitis/encephalitis through mechanisms like inhibiting bacterial cell wall synthesis, disrupting bacterial protein synthesis, interfering with nucleic acid synthesis, or damaging bacterial cell membranes. As a result, the concentration of bacteria may decrease when examined using the Biofire Filmarray tool, leading to false negative results.

Beyond the 14 pathogens that the Biofire Filmarray ME panel can identify, meningitis or encephalitis can be caused by various other pathogenic agents that are not detectable by the tool. These agents include bacteria such as Salmonella, Mycobacterium tuberculosis, Staphylococcus aureus, and Borrelia burgdorferi. Additionally, viruses like the West Nile virus, Japanese encephalitis virus, Flavivirus, and HIV cannot be detected by the Biofire Filmarray ME panel. Amoebas like Naegleria fowleri, fungi such as Coccidioides immitis, Aspergillus, Candida, and Mucormycosis, as well as parasites like Angiostrongylus cantonensis, Baylisascaris procyonis, and Gnathostoma spinigerum are also beyond the detection capabilities of the Biofire Filmarray ME panel.

Therefore, it must be acknowledged that the tool has limitations in identifying all potential causative agents of meningitis or encephalitis.

**Comparison of CSF Analysis with Biofire Filmarray**

There was 1 case with CSF analysis conclusions indicating viral etiology, matched by Biofire Filmarray results indicating a virus (Cytomegalovirus). There were 9 cases with CSF analysis conclusions suggesting bacterial infection, 4 cases suggesting partially treated bacterial infection, but the corresponding Biofire Filmarray results were negative. The McNemar test concluded with an asymptotic significance value of 0.000 (<0.05), indicating a difference between the results of CSF analysis and the Biofire Filmarray ME panel examination.

**Limitations of the Study**

The limitations of this study include the inability of the Biofire Filmarray ME panel to detect all pathogens causing meningitis/encephalitis. The Biofire Filmarray can...
only recognize 14 types of pathogens (comprising certain bacteria, viruses, amoebas, fungi, and parasites), while the etiology of meningitis/encephalitis encompasses more than just 14 types. Another limitation is the absence of serum glucose data during CSF sample collection and the opening pressure of CSF, thus the interpretation table of CSF analysis from Ropper et al. 2005 might be less accurate. During the eclipse phase, laboratory modalities cannot detect pathogens, especially if they are still present in tissues and have not spread to the CSF.

**CONCLUSIONS**

This study shows that there is a significant difference between the results of the CSF analysis and the Biofire FilmArray ME panel. Although the Biofire FilmArray may give false positive or false negative results in some situations, it cannot replace a broader medical interpretation and the professionalism of a Neurology Specialist in making a final diagnosis. Limitations of tools and methodologies must be taken into account in the interpretation of examination results.

**ACKNOWLEDGEMENTS**

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**AUTHOR’S CONTRIBUTION**

All authors contributed to this study’s conception and design, data analysis and interpretation, article drafting, critical revision, final approval, and data collection.

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**CONFLICT OF INTEREST**

All authors declare no conflicts of interest.

**ETHICAL CONSIDERATION**

This research has been officially approved by the Health Research Ethics Committee of dr. Soetomo Hospital Surabaya No. 0598/KEPK/II/2023, and Health Research Ethics Committee, Faculty of Medicine, Airlangga University No. 126/EC/KEPK/FKUA/2023

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