



Contribution of the Syndromic Approach in the Diagnosis of Meningitis at the University Hospital of Marrakech

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Abstract

Introduction: Central nervous system infections such as meningitis and encephalitis currently represent a real diagnostic and therapeutic challenge.

The objective of this study is to evaluate the contribution of the syndromic approach in the rapid and targeted diagnosis of meningitis and meningoencephalitis at the University Hospital of Marrakech.

Materials and Methods: This is a prospective study conducted over a period of one year (February 2018 - February 2019), including all patients with lumbar puncture with a cytology greater than 5 elements/mm³ and patients admitted for suspicion of meningoencephalitis.

Results: During this period, 176 lumbar punctures were performed that met the criteria to multiplex PCR, 101 cases for suspicion of meningoencephalitis and 75 cases for suspicion of meningitis.

The etiology was confirmed in 23% of the LCS treated for suspicion of meningitis. Bacterial etiology dominated the pattern in 70.5% of cases followed by viral (23.5%) and fungal (6%) etiology.

The etiology was confirmed in 14% of the LCS treated for suspicion of meningoencephalitis. Viral etiology dominated the pattern in 81% of cases followed by bacterial (12.5%) and fungal (6%) etiology.

Conclusion: Their rapid and efficient diagnosis made it possible to set up an appropriate treatment by avoiding the unnecessary prescription of antibiotics.

Keywords: Meningitis; Encephalitis; Multiplex PCR

Introduction

Infections that involve the Central Nervous System (CNS), such as Meningitis/Encephalitis (ME), are severe clinical conditions associated with high rates of morbidity and mortality as well as significant long-term sequelae [1]. ME may be caused by a wide variety of pathogens, including bacteria, viruses and fungi; clinical symptoms may vary (e.g. fever, headache to altered consciousness, neck stiffness and seizures) and often overlap with various infectious agents [1,2].

Early identification of ME causative pathogens has been proven to enable timely and appropriate treatment there by reducing death or permanent neurological damage (such as problems with vision and hearing, cognitive deficits, seizures and behavioral changes) [3,4].

Cerebrospinal Fluid (CSF) analysis is crucial in the diagnosis of CNS infection. Currently, in order to identify a potential causative pathogen, microbiological diagnosis in combination with cellular and chemistry parameters in CSF (some findings may suggest the general category of the causative agent, e.g. bacterial versus viral or fungal) are evaluated [4]. In particular, traditional tests such as Gram Stain (GS) with culture and pathogen-specific molecular method on CSF samples are used for the diagnosis of acute bacterial/fungal and viral CNS infections, respectively.

However, this conventional approach is conditioned by a low diagnostic yield and slow turnaround time. The sensitivity of GS and culture is relatively low and could further be reduced in patients who have received empiric therapy [3]. In addition, in ME cases, an etiology is not always identified. This could also be due to the lack of targeted testing and the low volume of CSF samples [1,5]. To overcome these limitations, interest focused on the development of standardized molecular

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diagnostic tests for simultaneous detection of the most common agents of infectious ME requiring a small volume of CSF [6,7].

New strategies need to be implemented that help clinician with the initial therapeutic decisions, possibly sparing patient from unnecessary anti-infective treatment. One strategy is the multiplex PCR test which-with pre-manufactured kits-facilitates the rapid identification of a variety of infectious agents.

Here we present our experience after 1 year of usage of novel multiplex PCR in the routine clinical setting of suspected Central Nervous System (CNS) infection. The aim of the study was to assess changed diagnostic and therapeutic procedures and evaluate the consequences of the introduction of FilmArray[®] multiplex PCR. In addition, we wanted to identify the most effective way to use this new technique in the routine patient management.

Materials and Methods

Study design and population

This prospective study was performed between February 2018 and February 2019, at Marrakech University Hospital Center.

The suspected cases of acute meningitis were identified by a clinician, based on the following criteria: Acute onset of fever (usually >38.5°C rectal or 38.0°C axillary), headache and one of the following signs: neck stiffness, altered consciousness or other meningeal signs [7]. Newborns were enrolled in the study if the newborn has a fever accompanied by nonspecific symptoms (e.g., poor feeding, vomiting, and diarrhea, rash) [8].

Multiplex PCR was performed for patients with a meningitis suspicion with cytology greater than 5 cells/mm³ and a sterile CSF culture after 24 h.

For patients admitted for suspicion of meningo-encephalitis, a PCR is performed if there is a strong clinical suspicion of encephalitis and if there are other biological abnormalities: procalcitonin, CRP, white blood cell count (Figure 1).

Multiplex PCR

Multiplex PCR on all samples was performed according to the manufacturer's protocol (BioFire; FilmArray[®] Meningitis/Encephalitis (ME) PCR Panel) [9]. The CSF panel was used to detect 14 pathogens: *CMV*, *enterovirus*, *HSV 1/2*, *HHV-6*, *human parechovirus*, *VZV*, *Cryptococcus neoformans/gattii*, *Escherichia coli K1*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae* (GBS) and *Streptococcus pneumoniae*. The results were available approximately 75 min after the start of the assay in the bacterial laboratory. The transport of the samples from the clinical wards to the laboratory usually took about 10 min.

Statistical analysis

All statistical analysis was performed with SPSS version 11.5.

Results

During the study period 1181 CSF was treated, the conventional method allowed the diagnosis of 71 bacterial meningitis with a positive CSF culture, and 1110 LCR had a sterile culture.

A total of 176 lumbar punctures were performed that met the criteria to multiplex PCR, 101 cases for suspicion of meningo-encephalitis and 75 cases for suspicion of meningitis.

Table 1: Characteristics of the study population and specimens analyzed.

The demographic characteristics	N. of patients
Age of patients:	
Adult	117
Pediatric	59
Gender:	
Male/Female	93/83
Immune status:	
Immunocompromised/Immunocompetent patient	28/148
Origin unit of specimens:	
Infectious Disease Unit	84
Intensive Care Unit	39
Emergency Unit	26
Pediatric Unit	22
Neurology Unit	5

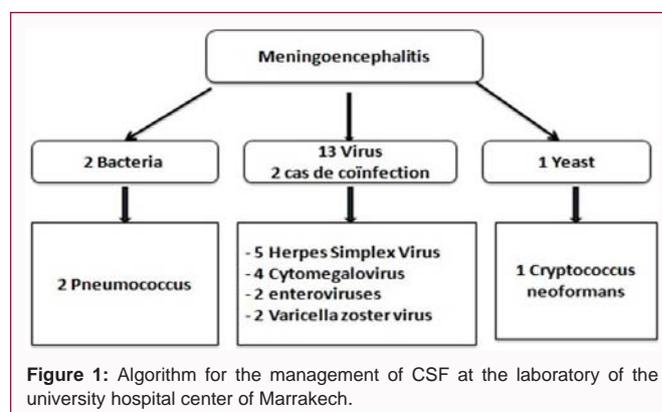


Figure 1: Algorithm for the management of CSF at the laboratory of the university hospital center of Marrakech.

The demographic characteristics

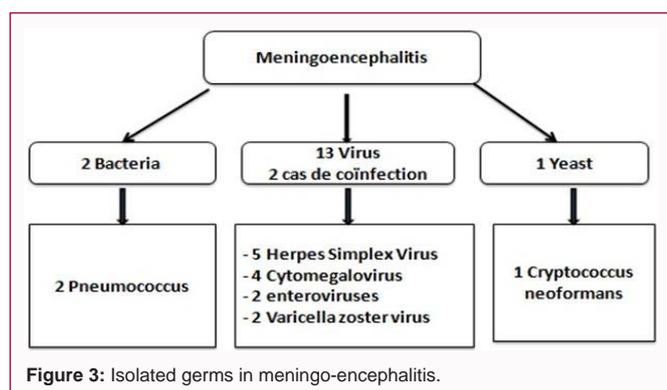
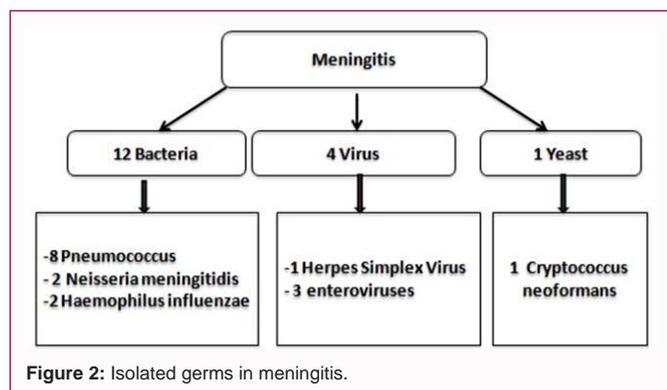
93 (53%) were from male patients and 83 (47%) were from female patients. Of all the 176 cases, 59 (34%) were of the age group <15 years, 110 (62%) were 15 to 64 years, and 7 (4%) were >65 years, 16% of patients were immunocompromised (HIV+). The demographic and laboratory characteristics of patients were included in this study are shown in Table 1.

Of the 176 cerebrospinal fluids tested, 31 (18%) yielded a positive result by multiplex PCR, 17 cases for suspicion of meningitis and 14 cases for suspicion of meningo-encephalitis.

The etiology was confirmed in 23% of the LCS treated for suspicion of meningitis. Bacterial etiology dominated the pattern in 70.5% of cases followed by viral (23.5%) and fungal (6%) etiology. *Pneumococcus* took first place with 47% of cases Figure 2.

The etiology was confirmed in 14% of the LCS treated for suspicion of meningoencephalitis. Viral etiology dominated the pattern in 81% of cases followed by bacterial (12.5%) and fungal (6%) etiology. Herpes simplex virus dominated the profile with 36% of cases Figure 3.

Positivity rate The FilmArray[®] ME Panel detected at least one pathogen in 31 of the 176 specimens that were tested, yielding an overall positivity rate of 18%, as shown in Table 2. The highest detection rates were in 15 to 34 age group and in the pediatric group (2 to 15 years). The most prevalent organisms detected during this study were *S. pneumoniae* (n=10), *Haemophilus influenzae* (n=2),



Neisseria meningitidis (n=2), *Enterovirus* (n=5), *Cytomegalovirus* (n=4), *HSV 1* (n=3), *HSV 6* (n=1), *HSV 2* (n=2), *Varicella Zoster Virus* (n=2), and *Cryptococcus neoformans* (n=2). Co-detections were observed in two cases, the first case for VZV and HVS6 in a 1-month-old infant with varicella lesions who was contaminated by his 7-year-old brother who had chicken pox, and the second case for CMV and HVS6 in a positive HIV patient.

Discussion

Meningitis and encephalitis often present with similar symptoms and the causative agents underlying the disease cannot be identified based on the clinical symptoms alone [10]. Diagnosis of meningitis and encephalitis has always been a challenge to laboratory managers and clinicians. Currently available testing methods are time-consuming and lack sensitivity and specificity. They are technically complex technologies and also require scientific expertise. Their accuracy may be affected by antibiotic administration prior to the testing, which is overcome by the FilmArray[®] technology. Also the small volumes of CSF obtained lead to a major challenge in the testing laboratory. Differentiation between bacterial, viral, or fungal meningitis/encephalitis is another challenge for clinical laboratories. While pleocytosis in the CSF is a sensitive marker of inflammation, several studies have shown that cell counts may be normal in both adult and pediatric patients despite an ensuing diagnosis of bacterial meningitis [11,12]. It is relevant to identify the etiologic agent in patients suffering from CNS infections. This would facilitate in identifying the most likely causative organisms. Diagnosis based on nucleic acid *in vitro* amplification-based molecular methods has a broader and superior application in clinical microbiology practice.

The Filmarray[®] ME Panel is an US-FDA approved de novo technology, based on the principle of multiplex PCR with detection by melting curve analysis. It adequately detects significant number

of targets and has high sensitivity and specificity compared to conventional techniques [13].

We analyzed clinical and microbiological data from prospectively recruited patients with suspected meningitis in a teaching hospital in Marrakech. The study was performed within the routine clinical and laboratory settings of a hospital that had very limited prior experience with molecular techniques. In addition to the conventional laboratory investigations used in the hospital, a simple and rapid molecular diagnostic system was introduced to enhance laboratory diagnostics during the study period. To the authors' knowledge, this is the first time a definite etiological diagnosis of viral meningitis has been made in patients in a public health facility in Marrakech.

In this study, the overall positivity rate observed with the FilmArray[®] ME Panel was similar to those described in other studies [13]. The etiology was confirmed in 14% of the LCS treated for suspicion of meningoencephalitis and in 23% of the LCS treated for suspicion of meningitis.

Virus were the most common etiological agents of CNS infections in this study with Enterovirus being the most common, followed by CMV and HSV-1 and VZV. Although the etiology is likely to vary between age groups and local epidemiology, this is in agreement with a study from Finland that found enteroviruses, followed by HSV-2 and VZV to be major causes of aseptic meningitis in adults [14] and a study from Brazil that reported enterovirus as the most common cause of meningitis, followed by HSV-1, cytomegalovirus and dengue virus [15].

Early detection of etiologic agents improves the outcome of meningitis [16] and adequate laboratory diagnostics are imperative. Culturing of bacterial and fungal agents takes time and has low sensitivity, as illustrated by the fact that no one of 14 CSF samples with potentially cultivatable organisms was culture positive.

The sensitivity of culture is affected by many factors including prior administration of antibiotics, suboptimal culturing conditions and media, and fastidious nature of some of the bacterial agents. Because of this and the unavailability of viral detection, virtually all patients with suspected meningitis in Ethiopia are treated as bacterial meningitis cases and the diagnosis is rarely re-evaluated over the course of the disease. This over-diagnosis of bacterial meningitis inevitably leads to an overuse of antibiotics. Hence, rapid molecular diagnostics can have a major impact in low income settings by increasing the likelihood of reaching a correct diagnosis and enabling correct patient management. This is crucial not only for the outcome of the individual patient, but also for hospital biosecurity measures, public health decisions and both local and global efforts to reduce and improve antimicrobial usage [17].

The introduction of syndromic testing of infectious diseases and fully automated multiplexed analyses represents a paradigm shift in microbiological diagnostics [18-20]. The FilmArray was able to detect microorganisms in 31 samples using the ME panel. Hence, such systems can improve patient management in settings with limited laboratory facilities. The FilmArray system was very easily implemented into a modestly equipped laboratory where personnel had little prior experience with molecular diagnostics.

However, there are a number of limitations to the sustained use of such automated systems in low-income countries. In Morocco, procurement of the necessary consumables is a complicated and

Table 2: Positivity rate for the FilmArray ME Panel for all samples and by age group.

		<2 months	2–23 months	2–15 years	15–34 years	35–64 years	>65 years
Bacteria							
<i>S. pneumonia</i>	10	0	2	2	3	2	1
<i>H. influenza</i>	2	0	0	2	0	0	0
<i>N.meningitides</i>	2	0	0	2	0	0	0
Viruses							
<i>Enterovirus</i>	5	1	2	2	0	0	0
<i>CMV</i>	4	0	0	0	4	0	0
<i>VZV</i>	2	0	1	0	0	1	0
<i>HSV 1</i>	3	0	0	1	1	1	0
<i>HSV 2</i>	1	0	0	0	1	0	0
<i>HSV 6</i>	2	0	1	0	1	0	0
Yeast							
<i>Cryptococcus neoformans</i>	2	0	0	0	0	2	0

lengthy process. The main obstacle, however, is the cost. Currently, the reagents needed for the analysis of one sample exceed 100 USD. Needless to say this is not sustainable in a public health system that is already financially constrained. On the other hand, a full course of treatment for suspected bacterial meningitis for 10 to 14 days [21-23], including only direct expenses for a hospital stay, routine investigations and antibiotic treatment, is likely to amount to more than 100 USD, even in Morocco. Another possible limitation of the system is the predefined selection of the pathogens in the panels. The panels were developed for an American market and may not be equally suited for Africa where other pathogens including *M. tuberculosis* are major cause of infections. It is also important that clinicians have a good understanding of test characteristics, interpretation of results and test limitations. Although lower than for conventional PCR, there is still a potential for sample contamination when using the FilmArray and the assay may detect latent or reactivated viruses [24,25]. The assays should be used with care and the positivity rates should be monitored.

The FA ME Panel test cannot supersede conventional microbiological procedures as it does not provide any information on antibiotic susceptibility. In addition, for virological analytes, quantitative results should be provided by the following singleplex quantitative PCR to evaluate in the clinical context the relevance of virus detected [26,27].

The implementation of the microbiological diagnostic workup with sensitive and specific FA ME Panel testing may improve the management of patients with suspected CNS infection by early specific treatment and may be especially useful in cases that require effective prevention measures such as post-exposure prophylaxis of close contacts.

Conclusion

This study highlights the importance of this syndromic approach in the rapid confirmation of the etiologies of community meningitis and meningoencephalitis at the University Hospital of Marrakech. Their rapid and efficient diagnosis made it possible to set up an appropriate treatment by avoiding the unnecessary prescription of antibiotics. In addition, its reasoned use can make it possible, at no extra cost, to improve the management of patients.

What is already known on this topic

The Filmarray[®] ME Panel is an US-FDA approved de novo technology, based on the principle of multiplex PCR with detection by melting curve analysis. It adequately detects significant number of targets and has high sensitivity and specificity compared to conventional techniques.

What this study adds

The aim of the study was to assess changed diagnostic and therapeutic procedures and evaluate the consequences of the introduction of Film Array[®] multiplex PCR. In addition, we wanted to identify the most effective way to use this new technique in the routine patient management.

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