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The Results of Meningitis/Encephalitis Panel in Children with Suspected Central Nervous System Infections

Maha Zakariya Mohammed[1*], Marwa Ramadan Mohamed Abdelhalim[2], Hoda Ezz Elarab Abdel Wahab Garrad[2]

Departments of Pediatrics[1] and Clinical pathology[2], Faculty of Medicine, Ain Shams University

Corresponding author: Maha Z. Mohammed, ORCID 0000-0002-6098-561X

, Email: mahazakariya@med.asu.edu.eg, Mobile:002 01124215285

ABSTRACT

Background: Central nervous system (CNS) infection in pediatrics is a considerable cause of morbidity and fatality.
Objective: The aim of the current study was to evaluate the Allplex™ Meningitis Panel Assays (ME) as a rapid diagnostic method for CNS infections.
Methods: The study included 329 cerebrospinal fluid (CSF) samples from pediatric patients with suspected CNS infections. The samples were analyzed using the Allplex™ ME panel in the period between January 2020 and January 2022.
Result: Out of the 329 CSF samples, 21 (6.4%) cases were positive. The commonest viral pathogen detected was parvo B19 (n=11), followed by human herpes virus 7 (n=3) and human herpes virus 6 (n=2). Streptococcus pneumoniae (n=1) was the only bacterial pathogen detected in our cohort. One patient with parvo B19 encephalitis improved markedly after receiving methylprednisolone. Another patient with parvo B19 was diagnosed consequently as anti-N-methyl-d-aspartate (NMDA) receptor encephalitis.
Conclusion: The Allplex™ ME Panel test can rapidly detect 18 pathogens from CSF. In view of antibiotics misuse, ME Panel aids in diagnosis of CNS infection helping the clinicians in early management and shortening the length of hospital stay. Case by case clinical evaluation is mandatory to avoid unnecessary request of the ME panel test. Further testing -specially for CSF autoantibodies- is warranted in case of worsening or unsatisfactory improvement of the condition.
Keywords: Encephalitis, Meningitis, PCR panel, Pediatric.

INTRODUCTION

Pediatric meningitis and acute encephalitis are a large contributor to lengthy hospital stay, high mortality, significant morbidity and subsequent long-lasting neurological sequelae [(1)].

The incidence of acute bacterial meningitis is estimated to be 5–7/100,000 in western countries [(2,3)], while pediatric acute encephalitis syndrome in children was reported to be around 10.5–13.8/100,000 [(4)].

Regardless the causative organism, CNS infections can present with nonspecific symptoms including, fever, neck stiffness, disturbed conscious level, nausea and vomiting, headache, photophobia, cranial nerve palsies, rash, behavioral changes, focal weakness, and seizure [(2,3)].

Therefore, the causative organism cannot be determined based on clinical presentation only [(5)].

In case of clinical suspicion of CNS infection, a lumbar puncture (LP) is needed at the earliest opportunity -unless contraindicated- to reach a diagnosis. Results of cerebrospinal fluid (CSF) microbiology are crucial for identifying the pathogenic organisms, making an appropriate antimicrobial treatment decision, determining the length of therapy, and avoiding the use of empirical antibiotic therapy [(6-8)].

CSF culture is typically positive in cases of bacterial meningitis that have not received treatment. Nevertheless, it is anticipated that the yield of CSF culture will be low in developing countries, where pretreatment with antibiotics is widespread [(9)].

In resource-limited setting, early diagnosis of CNS infection is crucial to reduce mortality rate and avoid expenses needed for empiric antimicrobials and isolation procedures in healthcare facilities. The aim of the current study was to evaluate the new multiplex PCR panel in determining the microbiologic etiologies causing CNS infections in children.

PATIENTS AND METHODS

Study design

This retrospective observational study was conducted at Ain Shams University (ASU) Children hospital. The study included 329 pediatric patients who were admitted between January 2020 and January 2022 with suspected CNS infection, where lumbar puncture was performed as a part of the routine investigations. Demographic data, clinical picture and CSF results were collected from patients' records.

Methodology:

CSF samples were collected and sent to ASU laboratory to analyze CSF protein, and glucose levels, cytology and microbiological assessment.

Bacteriological cultures were done on blood agar (BD) and chocolate agar (BD) for 72 hours.

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Identification of organisms was based on Gram staining and Vitek2C (bioMérieux SA, Marcy l'Etoile, France).

CSF (≥ 500 µL) was processed by the Allplex™ meningitis panel assays (ME) according to the manufacturer's instructions. The detection limits were 10 to 50 copies/µL. Seegene Allplex™ meningitis panel, Seoul, South Korea is CE-IVD approved test (mark under European Union on In Vitro Diagnostics Medical Devices) that ensures simultaneous identification of 18 meningitis pathogens including 12 viruses (Adenovirus (AdV), Enterovirus (HEV), Human parechovirus (HPeV), Mumps virus (MV), Parvovirus B19 (B19V), Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Herpes simplex virus type 1 (HSV1), Herpes simplex virus type 2 (HSV2), Human herpes virus 6 (HHV6), Human herpes virus 7 (HHV7) and Varicella-zoster virus (VZV) and 6 bacteria (Escherichia coli K1 (E. coli K1), Group B streptococci (GBS), Haemophilus influenzae (HI), Listeria monocytogenes (LM), Neisseria meningitidis (NM) and Streptococcus pneumoniae (SP), using multiplex one step real time R-PCR.

Ethical consideration:

The study protocol gained approval from the local Ethics Committee of the Pediatric Department, Faculty of Medicine, Ain Shams University. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistics interpretation

The researcher checked the data, coded it, and analyzed it with SPSS version 24. Data were presented as number and percentage.

RESULTS

A total of 329 pediatric patients were included during the 24-month study period of the current study. Cerebrospinal fluid specimens were analyzed using the Seegene Allplex™ ME panels. One pathogen was detected in 21 out of 329 CSF samples, (Positivity rate= 6.4%), as shown in table 1).

Parvo B19 virus was the most common detected organism (n=11) 52%, followed by HHV7 (n=3) 14% and HHV6 (n=2) 10%. Only 1 positive result was detected for enterovirus, HSV1, mumps and EBV. Streptococcus pneumoniae (n=1) was the only bacterial pathogen detected in CSF in our cohort

Table 1 Results of pathogens detected by Allplex ME panel; presented as total number and percentage

	Number	Percentage
No pathogen detected	308	93.6%
Pathogen detected	21	6.4%
Bacteria	1	0.3%
Escherichia coli K1 (*E. coli* K1)	0	0
Group B streptococci (GBS)	0	0
Haemophilus influenzae (HI)	0	0
Listeria monocytogenes (LM)	0	0
Neisseria meningitidis (NM)	0	0
Streptococcus pneumoniae (SP)	1	0.3%
Viruses	20	6%
Adenovirus (AdV)	0	0
Enterovirus (HEV)	1	0.3%
Human parechovirus (HPeV)	0	0
Mumps virus (MV)	1	0.3%
Parvovirus B19 (B19V)	11	3.3%
Cytomegalovirus (CMV)	0	0
Epstein-Barr virus (EBV)	1	0.3%
Herpes simplex virus type 1 (HSV1)	1	0.3%
Herpes simplex virus type 2 (HSV2)	0	0
Human herpes virus 6 (HHV6)	2	0.6%
Human herpes virus 7 (HHV7)	3	0.9%
Varicella-zoster virus (VZV)	0	0

Table 2 shows the demographic characteristics, age, gender, patient admission place and the results of diagnostic tests for meningitis -in positive cases as detected by ME panel- such as TLC, CSF glucose and proteins, cell count, gram staining, and culture findings. Out of the 21 positive samples ten (48%) were from male patients and 11 (52%) from female patients. Among the 21 positive cases, twenty (95%) were viral, yet only one was bacterial (Table 2).

Onsite analysis showed 6/21 (29%) specimens showed more than 5 cells/µL, yet 3/21 (14%) were bloody samples (cannot be counted). Eight samples out of twenty-one (38%) showed increase in protein in CSF and glucose measurements were generally within the normal range. The bacterial cultures were negative in all samples even in Streptococcus pneumoniae.

Table 2 Summary of demographic data and results of blood and CSF tests in positive cases as detected by ME panel

				CSF					
S. No	**Age/sex**	**Location**	**Blood TLC**	**Glucose**	**Protein**	**Cell count**	**Gram stain**	**Culture**	**ME panel**
1	4 years/F	PICU	7	105	79	40	Neg	Neg	Parvo
2	8 years/M	Ward	8.5	63	40	0	Neg	Neg	Parvo
3	7 months/F	Ward	8	44	65	bloody	Neg	Neg	Parvo
4	7 months/F	Ward	7.7	53	37	0	Neg	Neg	HHV6
5	5 years/F	Ward	9	60	37	0	Neg	Neg	EBV
6	1 year/F	Ward	8	N/A	N/A	bloody	Neg	Neg	Parvo
7	13 years/F	Ward	10	62	59	10	Neg	Neg	HHV7
8	1 year/F	Ward	8.7	50	43	0	Neg	Neg	HHV6
9	5 days/F	NICU	10	65	215	45	Neg	Neg	Parvo
10	7 years/F	Ward	6.1	118	59	0	Neg	Neg	Parvo
11	11 years/M	Ward	8	79.7	34	0	Neg	Neg	HHV7
12	6 years/M	Ward	9	88	35.3	0	Neg	Neg	HHV7
13	6 years/F	PICU	15.1	78	44	0	Neg	Neg	Parvo
14	6 years/M	Ward	13.9	59	44	0	Neg	Neg	Parvo
15	1.5 years/M	Ward	6.4	72	23	0	Neg	Neg	HEV
16	5 years/M	Ward	2.7	55.8	178	25	Neg	Neg	Parvo
17	5 years/F	Ward	8	61	45	0	Neg	Neg	Parvo
18	7 months/M	Ward	12	44	818	278	Neg	Neg	Parvo
19	1 year/M	Ward	8.3	56	37	0	Neg	Neg	Mumps
20	9 months/M	Ward	10	N/A	N/A	bloody	Neg	Neg	HSV1
21	1 year/M	Ward	12.9	51	63	310	Neg	Neg	S. pneumoniae

M=male, F=female, PICU=pediatric intensive care unit, NICU=neonatal intensive care unit, Neg=negative, N/A=not applicable, CSF= cerebrospinal fluid, TLC= total leucocytic count, HHV= Human herpes virus, EBV= Epstein-Barr virus,HEV= Enterovirus, HSV1= Herpes simplex virus type 1, S. pneumoniae= Streptococcus pneumoniae

Interesting cases

It worth mentioning that one of our patients with parvo B19 encephalitis had pancytopenia on admission and the result of parvo B19 PCR was also positive in blood. The patient didn't show improvement on supportive treatment. Hence, methylprednisolone was commenced, and the patient improved markedly after receiving 3 doses on 30 mg/kg/dose.

Another patient with parvo encephalitis developed new neurological deficits in form of encephalopathy and abnormal shaking movement with behavioral changes, 21 days after initial improvement, immune encephalitis was suspected and testing for CSF Anti-NMDA antibodies was requested and turned out positive. Patient didn't respond to methylprednisolone or intravenous immunoglobulin (IVIG) but showed marked improvement after receiving rituximab. We are not sure if this patient was Anti NMDA encephalitis from the start or the parvo B19 infection was the trigger for Anti NMDA antibody mediated autoimmune encephalitis.

In our center, the UK CNS infection guidelines were followed since 2012 [(10)]. Due to limited resources, our center didn't support any testing for virus detection in CSF prior to 2019, so our policy was to keep the patient on acyclovir for at least 14 days. From 2019, the ME panel was introduced to our Laboratory at ASU. Allplex™ ME Panel results are obtained within 4-24 hours according to time of sample transport to the laboratory and if this is during weekdays or weekends. Herpes simplex was identified in only 1 patient during the study. Length of therapy of "empiric" acyclovir was significantly shorter for all patients in our study from 14 days to one day or maximum two days in weekends after introducing the ME panel. It is to be noted that the length of stay in hospital for our patients varied from 2 to 21 days according to the rate of improvement of patients' conditions.

DISCUSSION

The current study retrospectively investigated CSF samples, collected at ASU children hospital in Cairo, Egypt, using Allplex™ ME panel.

The positive rate of detection by Allplex™ ME panel was 6.4% (21/329). This relatively low figure in relation to the sensitivity of Allplex™ ME panel that

can detect 18 potential causative agents of CNS infection might be explained by several causes. Firstly, bacterial meningitis cases were referred to the fever hospital -for isolation- once clinically suspected. High index of suspicion and routine availability of the ME panel could result in over-requesting of the diagnostic test. Additionally, antibiotics misuse prior to admission might have reduced the rate of bacterial detection in CSF [9].

Our results were in agreement with **Tarai and Das** who reported that the positivity rate of FilmArray® ME Panel was 10.4% [11]. Similar findings were reported by **Radmard *et al***, who reported (6.4%) positive ME panel results in their study that included 705 adult and pediatric patients [12]. In contrary, **Säll and coworkers** reported identification of a likely pathogenic organisms of CNS infections in 23% of their cases using FilmArray ME [13].

In the current study, the most prevalent virus detected was parvo B19 virus (n = 11) 52%, followed by HHV7 (n = 3) 14%, HHV6 (n = 2) 10%. Only 1 positive result was detected for Enterovirus, HSV1, mumps and EBV.

On other hand, **Tarai and Das** reported that enterovirus was the most detected virus in their cohort (22.8%) in North India [11]. Several studies conducted in India, Kuwait, and European countries reported that EV was detected in around 21% of patients with encephalitis, while 12.9% of patients showed positive results for HSV-1 or HSV-2 [12]. In Nepal, **13. Säll *et al***, reported several cases of bacterial meningitis, the detected organisms included H. influenzae (n = 5) and S. pneumoniae (n = 4), and Neisseria meningitidis (n-1). In the same study, enterovirus was the most prevalent virus, it was detected in eight CSF samples [13].

CONCLUSION

The Allplex™ ME panel is one of the latest CE-IVD approved molecular technologies, which can rapidly identify 18 pathogens in CSF associated with the CNS infections. The early identification of viruses and bacteria will prevent unnecessary use of empiric antimicrobial therapy and decrease the cost of long hospital staying. Case by case clinical evaluation is mandatory to avoid unnecessary request of the ME panel test. Further testing -specially for CSF autoantibodies- is warranted in case of worsening or unsatisfactory improvement of the condition.

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REFERENCES

1. **Kliegman R *et al*. (2020)**: Central nervous system infection. Nelson Textbook of Pediatrics, 21st ed. Philadelphia: Elsevier, pp. 2904.
2. **Tacon C, Flower O (2012):** Diagnosis and management of bacterial meningitis in the paediatric population: a review. Emerg Med Int., 2012:320309.
3. **Brouwer M, Tunkel A, van de Beek D (2010)**: Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clin Microbiol Rev., 23:467–492.
4. **Jmor F, Emsley H, Fischer M *et al.* (2008**): The incidence of acute encephalitis syndrome in Western industrialised and tropical countries. Virol J., 5:134.
5. **Kupila L, Vuorinen T, Vainionpaa R *et al.* (2006):** Etiology of aseptic meningitis and encephalitis in an adult population. Neurology, 66: 75–80.
6. **Polage C, Cohen S (2016):** State-of-the-art microbiologic testing for community-acquired meningitis and encephalitis. J Clin Microbiol., 54: 1197–1202.
7. **van Ettekoven C, van de Beek D, Brouwer M (2017):** Update on community-acquired bacterial meningitis: guidance and challenges. Clin Microbiol Infect., 23: 601–606.
8. **Tan Y, Gill A, Kim K (2015):** Treatment strategies for central nervous system infections: an update. Expert Opin Pharmacother., 16: 187–203.
9. **Singhi S, Khetarpal R, Baranwal A, Singhi P (2004):** Intensive care needs of children with acute bacterial meningitis: a developing country perspective. Ann Trop Paediatr., 24: 133–140.
10. **Kneen R, Michael B, Menson E *et al.* (2012):** National Encephalitis Guidelines Development and Stakeholder Groups. Management of suspected viral encephalitis in children - Association of British Neurologists and British Paediatric Allergy, Immunology and Infection Group national guidelines. J Infect., 64(5):449-77.
11. **Tarai B,Das P(2019):** FilmArray® meningitis/encephalitis (ME) panel, a rapid molecular platform for diagnosis of CNS infections in a tertiary care hospital in North India: one-and-half-year review. Neurological Sciences, 40:81–88.
12. **Radmard S, Reid S, Ciryam P *et al* (2019)**: Clinical utilization of the FilmArray Meningitis/Encephalitis (ME) multiplex polymerase chain reaction (PCR) assay. Front Neurol., 10:281.
13. **Säll O, Thulin Hedberg S, Neander M *et al* (2019):** Etiology of central nervous system infections in a rural area of Nepal using molecular approaches. *Am J Trop Med Hyg*., 101(1):253-259.