



Original Article

## Neurovirology Panel Taqman PCR Test for Detection of Viral Pathogens in CSF – A Pilot Study

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### ABSTRACT

**Introduction:** Viral infections of the central nervous system (CNS) can lead to severe neurological complications, yet their diagnosis can be challenging due to limitations of conventional methods. PCR assays on CSF offer a sensitive and specific tool for rapid detection of viral pathogens. This study aimed to detect the viral pathogens qualitatively using multiplex CSF neuro viral panel PCR in patients presenting with suspected viral CNS infections.

**Material and Methods:** This study was conducted at VRDL lab, Government Medical College and Civil hospital Miraj, from November 2024 to June 2025. Patients with suspected viral CNS infections were analysed using a multiplex real-time PCR panel (TaqMan PCR kit) targeting a range of neurotropic viruses including HSV-1, HSV-2, CMV, EBV, VZV, HHV-6, HHV-7, Parvovirus B-19, Adenovirus, Enterovirus, and Parechovirus. Patient's demographics, clinical presentation, and other relevant diagnostic findings (e.g. CSF leukocyte count, protein levels) were collected and analysed in conjunction with the PCR results.

**Results:** Total 81 CSF samples were tested with the neuro viral panel PCR, out of which 37(46%) samples were positive for different viruses including HSV-1(13), Parvovirus B-19 (09), VZV (05), EBV (04), CMV (04), and HHV-6 (02). Results of 6 (7.4%) samples were inconclusive. Specifically, 88% of patients with a clinical diagnosis of viral meningitis/encephalitis had a positive PCR result, while 12% of patients not suspected to have viral CNS infections, were tested positive for EBV, CMV requiring cautious interpretation.

**Conclusion:** CSF neuro viral panel PCR is a valuable diagnostic tool for CNS viral infections. Optimizing its use requires consideration of clinical factors alongside laboratory results for improved diagnosis and management. Further research is warranted to establish specific algorithms that combine clinical information with PCR findings to maximize the utility of this diagnostic modality.

**Keywords:** Viral Meningoencephalitis, Multiplex PCR, Herpes simplex virus type 1.

### INTRODUCTION

Central Nervous System (CNS) infections are infections of the brain and spinal cord, and their surrounding membranes (meninges).

They are a spectrum of conditions that can affect the brain parenchyma, meninges, or spinal cord. They are considered potentially life-threatening until a definitive diagnosis is made.

The confident diagnoses of neuroviral infections will help to improve the management of infected people and facilitate

the development of specific antiviral compounds. It will help in aiding the development of methods to control disease. The diagnosis of CNS viral infections is challenging. It is often difficult to clinically distinguish between viral and bacterial CNS infections at presentation. Both types of meningitis can manifest with similar symptoms especially in children such as fever, headache, photophobia, and neck stiffness. In neonates and infants, the typical signs of meningitis may be absent.

Additionally, with the inclusion of conjugated *Streptococcus pneumoniae* and *Haemophilus influenzae* type B (Hib) vaccines in routine immunization programs, viruses have become the most common cause of meningitis worldwide [1]. Commonly used diagnostic parameters like cerebrospinal fluid (CSF) cytology, biochemical parameters and acute-phase reactants [e.g., C-reactive protein (CRP) and white blood cell count] are insufficient to reliably distinguish viral from bacterial meningitis [2].

The inability to determine the etiology early often leads to prolonged and potentially unnecessary use of antibiotics and antivirals, as well as extended hospital stays.

Molecular diagnostic tests based on polymerase chain reaction (PCR) -particularly multiplex PCR panels- allow rapid and reliable identification of multiple neurotropic pathogens in CSF samples. These tests enable timely and accurate pathogen identification and initiation of appropriate treatment.

Enterovirus and HPeV (Human Parechovirus) are frequent cause of meningitis and sepsis-like illness among young children. [5] Moreover, if a benign viral agent such as Enterovirus (EV) is identified, PCR can inform early clinical decision-making and potentially prevent unnecessary antimicrobial use [2-5].

The primary objective of this study is to evaluate the impact of CSF-PCR panel results on clinical decision making and patient management.

The secondary objective is to determine the frequency of viral agents detected by the CSF-PCR panel and to assess whether clinical or laboratory features could predict the likely pathogen.

## MATERIAL AND METHODS

- This study was conducted at VRDL lab in Government Medical College and Civil Hospital Miraj, from November 2024 to June 2025.
- This study included patients aged Day 1 to 75 years with suspected CNS infection in intensive care unit.
- Patients with clinically suspected viral CNS infections were analysed using a multiplex real-time PCR panel (TaqMan PCR kit) targeting a range of neurotropic viruses including HSV-1, HSV-2, CMV, EBV, VZV, HHV-6, HHV-7, Parvovirus B-19, Adenovirus, Enterovirus, and Parechovirus.
- CSF was collected in sterile container by lumbar puncture technique under strict aseptic conditions. It is transported immediately to the laboratory for processing.
- Patients having non-infectious reasons (e.g., suspected intracranial hypertension, evaluation of seizures, metabolic workup, Guillain-Barre syndrome, autoimmune or vasculitic disease, malignancy, subarachnoid haemorrhage), and those with ventriculoperitoneal shunts were excluded.
- Patient's demographics, clinical presentation, and other relevant diagnostic findings (e.g. CSF cytology, biochemical analysis), acute phase reactant levels were collected and analysed in conjunction with the PCR results.
- The clinical data were obtained by clinicians, detailed information was collected regarding the clinical episode associated with the CSF sample. The clinical findings at the time of presentation including: fever ( $>37.5^{\circ}\text{C}$ ), headache, meningism, seizures, rash, focal neurological signs, altered level of consciousness, immunosuppression, as well as the treating physician's discharge diagnosis were entered into a standardised proforma.
- Altered level of consciousness was defined as a Glasgow Coma Score  $\leq$  14, or in infants the record of drowsiness. Patients were recorded as immunocompromised if they were one or more of the following: HIV seropositive, receiving chemotherapy, a transplant recipient, or were documented as having an inherited immunodeficiency.
- The results of CNS imaging (computed tomography, magnetic resonance imaging, and in infants, cranial ultrasound) as well as electroencephalography (EEG) performed during the hospital admission were recorded as normal or abnormal.
- CSF white cell count (WCC), total protein, and culture results were recorded for each CSF sample. The WCC and protein levels were categorised as normal or abnormal using age related normal values.
- For CSF analysis, pleocytosis was defined as  $>16$  leukocytes/mm<sup>3</sup> for neonates, 10 leukocytes/mm<sup>3</sup> for infants aged 1-3 months and  $>5$  leukocytes/mm<sup>3</sup> for children older than 3 months and adults. Elevated CSF protein was defined as  $>100$  mg/dL for neonates,  $>75$  mg/dL for infants aged 1-3 months and  $>45$  mg/dL for children older than 3 months

and adults. Low CSF glucose was defined as <40 mg/dL for neonates, infants aged 1-3 months, and <50 mg/dL for children older than 3 months and adults.[6]

The likelihood of central nervous system (CNS) infection was classified as likely, possible, or unlikely.

**Table no. 1 Classification of CNS viral infections using clinical and laboratory criteria**

**Likely CNS viral infection (i and/or ii):**

- i. Raised CSF white cell count (defined by age group) accompanied by one or more of the following: meningism, headache, or fever (>37.5°C) (with no other explanation)
- ii. Altered level of consciousness or focal neurological signs accompanied by fever (>37.5°C) or headache (with no other explanation)

**Possible CNS viral infection:**

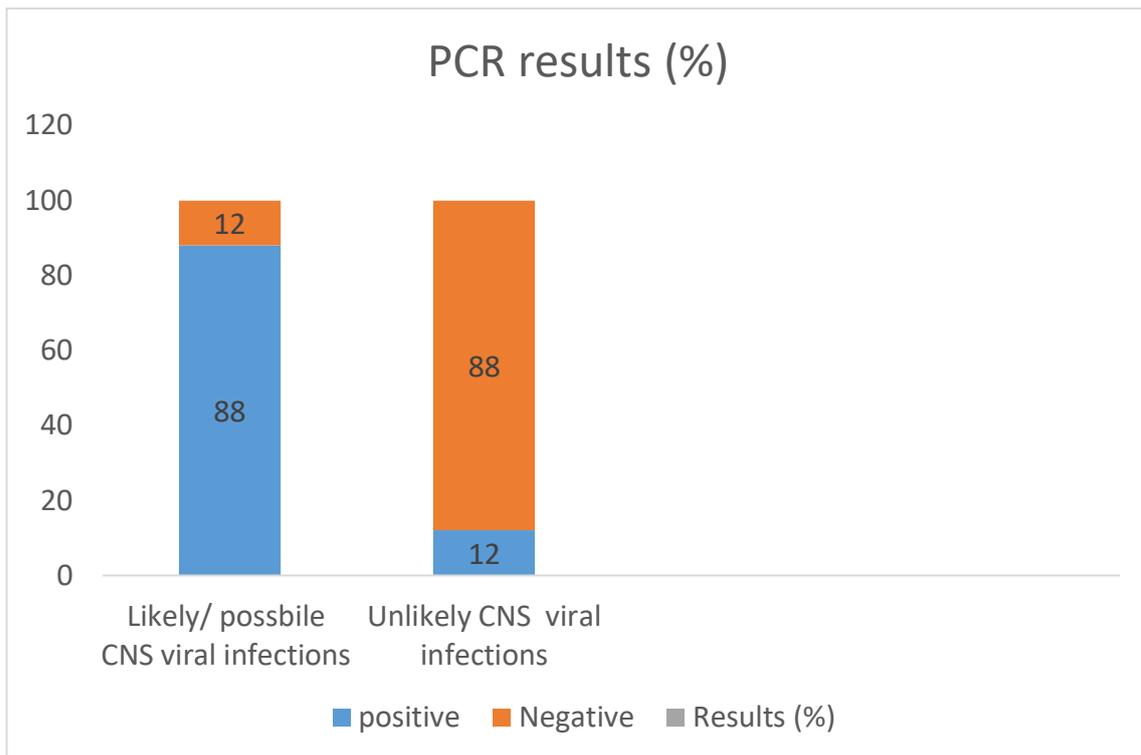
Attending physician's final diagnosis of a viral CNS infection with any combination of signs or symptoms

**Unlikely CNS viral infection (one of the following):**

- i. Another definite diagnosis (for example, multiple sclerosis or bacterial meningitis)
- ii. Non-specific diagnosis (for example, febrile convulsion where the clinical or laboratory features were not consistent with the above categories)
- iii. No definite diagnosis (for example, fever of unknown cause where the clinical or laboratory features were not consistent with the above categories).

-\*Classification adapted from Jeffery et al.[7]

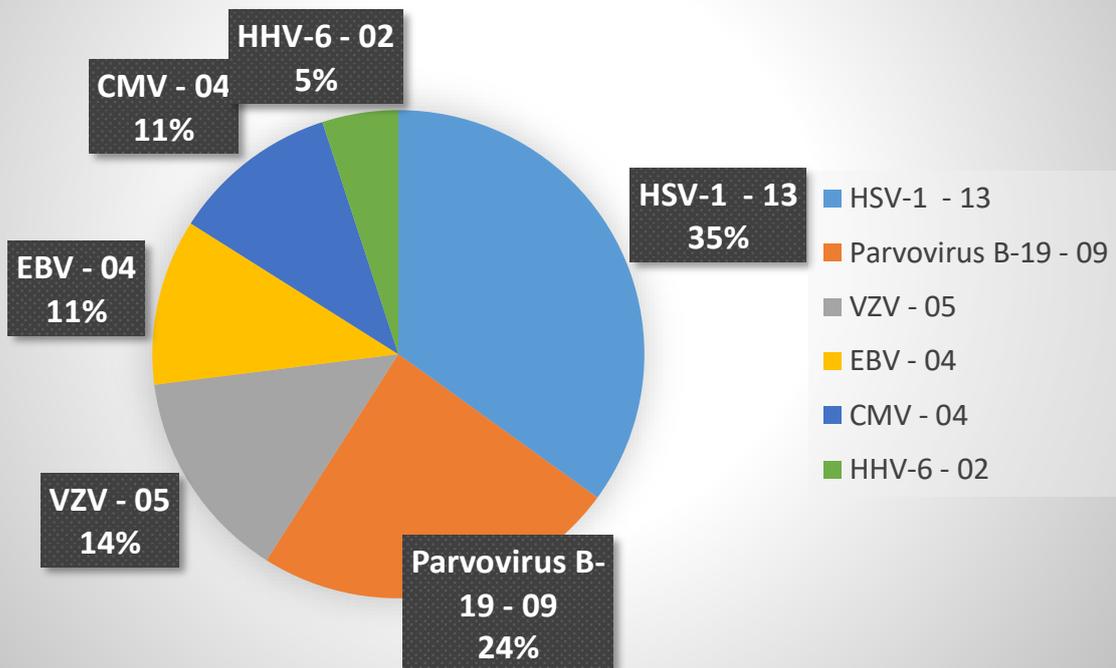
**RESULTS**



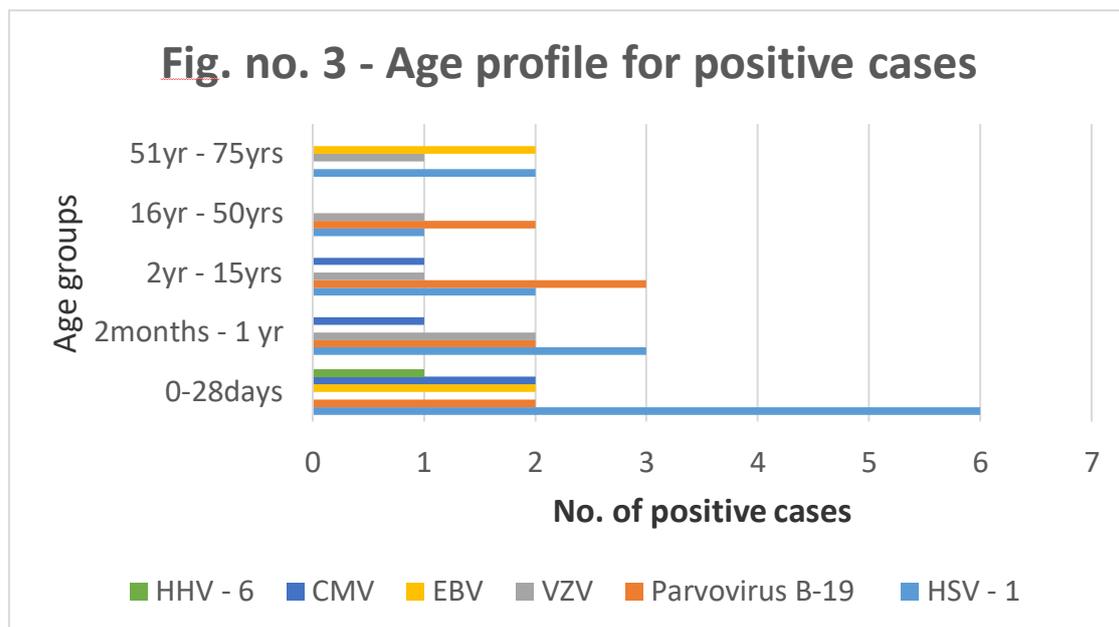
**Figure no. 1**

- Total 81 CSF samples
- 37(46%) -Positive
- 38(47%) - Negative
- 6 (7%) - Inconclusive.

**Fig.no. 2 -Distribution of viral agents  
(N- 37)**



**Fig. no. 3 - Age profile for positive cases**



We have processed total 81 samples, out of which in 37 (47%) samples virus was detected. HSV-1 was found in 13 samples, Parvovirus B-19 detected in 9 samples, Varicella zoster virus in 5 samples, Epstein - Barr virus and cytomegalovirus detected in 4 samples each. In 2 samples found Human herpes virus-6. Maximum no. of positive results came from neonate and infants.

Specifically, 88% of patients with a clinical diagnosis of viral meningitis/encephalitis had a positive PCR result, while 12% of patients not suspected to have viral CNS infections, were tested positive for EBV, CMV requiring cautious interpretations.

- Proportion of CSF samples with abnormal clinical or laboratory findings in which virus was detected by PCR

| Symptom, sign, or lab. investigation | Finding present | Finding absent |
|--------------------------------------|-----------------|----------------|
| Fever                                | 30              | 07             |
| Meningism                            | 34              | 03             |
| Headache                             | 28              | 09             |
| Rash                                 | 11              | 26             |
| Seizures                             | 31              | 06             |
| Immunosuppression                    | 08              | 29             |
| Abnormal CNS imaging                 | 34              | 03             |
| Abnormal EEG                         | 12              | 25             |
| CSF pleocytosis (lymphocytosis)      | 35              | 02             |
| Elevated CSF protein                 | 28              | 09             |
| Focal neurological deficits          | 13              | 24             |
| Leucocytosis                         | 29              | 08             |
| Elevated CRP                         | 13              | 24             |
| Decreased CSF glucose                | 01              | 36             |

### Interpretation

- **Highly Significant Findings (p < 0.001):** Fever, Meningism, Seizures, Abnormal Imaging, and CSF Pleocytosis are the most dominant clinical features in our study.
- **Non-Significant Findings (p > 0.05):** Focal neurological deficits and Elevated CRP do not show a statistically significant difference between presence and absence in this study.
- A p-value of < **0.0001** indicates that the distribution of these findings is **statistically significant**. The high frequency of elevated protein compared to the rare occurrence of decreased glucose (1 case) suggests a specific diagnostic pattern, often seen in **viral or aseptic meningitis** where glucose levels typically remain normal.
- Proportion of CSF samples with abnormal clinical or laboratory findings in which virus was **not detected** by PCR

| Symptom, sign, or lab. Investigation | Finding present | Finding absent |
|--------------------------------------|-----------------|----------------|
| Fever                                | 27              | 11             |
| Meningism                            | 08              | 30             |
| Headache                             | 06              | 32             |
| Rash                                 | 07              | 31             |
| Seizures                             | 04              | 34             |
| Immunosuppression                    | 00              | 38             |
| Abnormal CNS imaging                 | 17              | 21             |
| Abnormal EEG                         | 14              | 24             |
| CSF pleocytosis (lymphocytosis)      | 03              | 35             |
| Elevated CSF protein                 | 07              | 31             |
| Focal neurological deficits          | 11              | 27             |
| Leucocytosis                         | 14              | 24             |
| Elevated CRP                         | 19              | 19             |
| Decreased CSF glucose                | 13              | 25             |

- **Interpretation:**
- **Highly Significant:** Fever, Meningism, and Headache show a p-value < **0.05**, suggesting these are strong markers in this study population. CSF Pleocytosis and elevated CSF protein show a p-value < **0.0001** which is highly significant. Furthermore, decreased CSF glucose show a P value 0.0298 which is significant.
- **Non-Significant:** Features like **Elevated CRP (p=1.000)** and **Abnormal CNS Imaging (p=0.638)**, leucocytosis (**p=0.1010**) show no statistical significance in this study.

Fig. no. 4 - Impact on patients treatment based on PCR results(%)

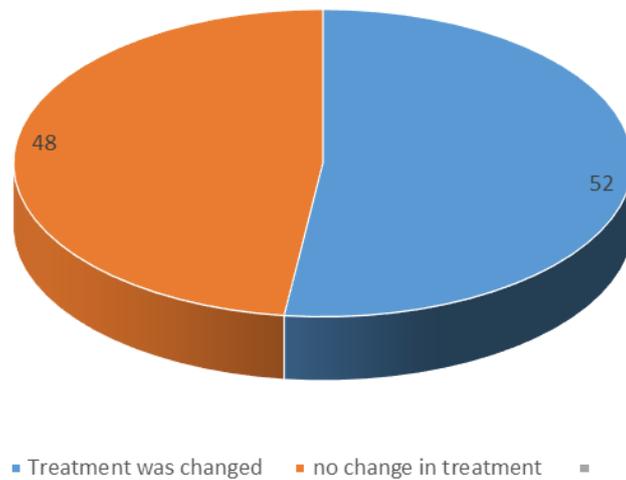
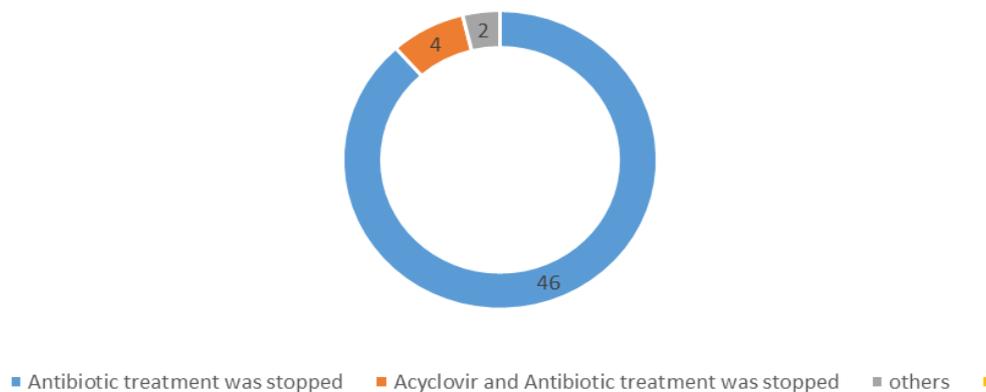


Fig. no. 5 Treatment changes based on PCR results(%)



- In this study, the CSF neuroviral PCR panel demonstrated a treatment change for 48% of patients, amongst which antibiotic treatment was stopped in 46% patients and patients were started on Acyclovir. Acyclovir and antibiotic treatment was stopped in 4% cases. Conversely, in 52% of cases, treatment remained unchanged.

#### DISCUSSION

- **Positivity Rate (46%):** positive yield in our study, aligns more closely with studies targeting high-risk symptomatic patients, such as a 2022 study where **51.7%** were positive[8], or specific 2025 cohorts reporting **56.9%** positivity.[9]
- **Negative Rate (47%):** It is reflecting either the panel's role in not identifying pathogens outside the standard multiplex targets. Most multi-centre studies report higher negative rates, often exceeding **60–75%**, particularly when the panel is used as a screening tool rather than for highly suspicious cases. [10,11]. Multiplex panels showed NPV of over 99%, meaning a negative result is highly reliable for ruling out the 14 targeted pathogens.[17]

- **Inconclusive Rate (7%):** inconclusive rate of 7% is slightly above the typical range. For comparison, some studies report a diagnostic discrepancy or "low threshold" inconclusive rate of roughly **3–5%**, often due to viral loads falling below the limit of detection (e.g., <250-500 copies/mL).[7,8,10]
- **HSV-1 (13 positive):**  
HSV-1 is the most common cause of infectious encephalitis. In diagnostic studies, BioFireFilmArray and similar multiplex panels show high specificity (>99%) but variable sensitivity for HSV-1 (roughly 84–97%). A positive result typically indicates active infection, though low levels (high cycle thresholds) can sometimes be found in early disease. [12]
- **Parvovirus B19 (09 positive):**  
CSF positivity for Parvovirus B19 is rare, with 2026-era studies reporting rates as low as **0.8% to 2.2%** among suspected meningitis cases. While rare, it is increasingly recognized as a cause of meningoencephalitis in both children and adults, particularly during outbreaks. [13]
- **VZV (05 positive):**  
VZV is a leading cause of viral meningitis and may occur without a classic shingles rash. Studies show VZV DNA in roughly **5% to 10%** of neurological cases. Positive VZV PCR in CSF is highly significant even at low levels, as it can be associated with delayed neurological complications like stroke. [14]
- **HHV-6 (02 positive) & EBV/CMV (04 positive):** HHV-6 is frequently the most common pathogen detected in some multiplex panels, appearing in up to **15.3%** of cases. Detection of HHV-6, EBV, and CMV must be interpreted with caution. These viruses often remain latent in the body and may "reactivate" or simply be present at low levels without being the primary cause of the current illness, especially in immunocompromised individuals.[15]
- **EBV and CMV** are more common in adults is strongly supported in the context of specific populations. In adults, these viruses are frequently detected in those who are **immunocompromised** (e.g., HIV-positive). For instance, a study of adults with aseptic meningitis found **EBV** in 50% and **CMV** in 14% of cases.[15]
- While EBV and CMV are common in adults, they are also prevalent in children, particularly in high-density or lower-income environments. A 2024 study in China noted that EBV and CMV are both prevalent in children, with CMV positivity peaking between 28 days and 1 year of age. [16]
- Patients clinical symptoms and laboratory feature findings are consistent with studies by Jeffery KJ, Read SJ, Peto TE, et al and Sabine E. Olie, Christian Ø. Andersen, et al [8,11]<https://journals.asm.org/doi/full/10.1128/cmr.00021-24-con3>.

#### Impact of PCR results on patients' treatment

- In this study, the CSF neuroviral PCR panel demonstrated a treatment change for 48% of patients, amongst which antibiotic treatment was stopped in 46% patients and patients were started on Acyclovir. Acyclovir and antibiotic treatment was stopped in 4% cases. These findings underscore the panel's value in reducing the duration of empiric therapy. A study by Buchan et al showed that there was treatment change for 30-50% based on PCR results.[19]
- Another study by Murien Cailleaux et al showed, there was treatment change for 48% of patients, amongst which antibiotic treatment was stopped in 42% of patients.[11]
- **Support of Empiric Choices:** In our study the 52% of unchanged treatments often reflect the test's role in **confirming** current management rather than failing it. A 58% "no change" result can provide clinicians with the confidence to continue an existing regimen if the clinical suspicion remains high despite a negative PCR.[11]
- **Diagnostic Stewardship:** In our study 66 patients having normal CSF parameters (low WBC, normal protein) showed negative PCR results. Research suggests that many negative results occur in patients with normal CSF parameters (low WBC, normal protein), indicating that testing could be more selectively applied to improve cost-effectiveness. [18]

#### Study Limitations

This study has some limitations. It was a single centre study with a relatively small sample size, and we did not perform confirmatory testing (e.g. sequencing or separate PCR assays) for the pathogens detected by the panel. This study cannot differentiate between the active infection and reactivation of latent infection.

## CONCLUSION

- CSF neuro viral panel PCR is a valuable diagnostic tool for CNS viral infections.
- Optimizing its use requires considering clinical factors alongside laboratory results for improved diagnosis and management.
- Our findings indicate that PCR results enabled the discontinuation of unnecessary antimicrobial treatments in many cases, thereby shortening treatment durations and potentially reducing hospital stays and healthcare costs. Wider implementation of rapid multiplex PCR panels may help to improve the management and outcomes of CNS infections.
- Further research is warranted to establish specific algorithms that combine clinical information with PCR findings to maximize the utility of this diagnostic modality.

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## DECLARATION

Conflicts of interests: The authors declare no conflicts of interest.

Author contribution: All authors have contributed in the manuscript.

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