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1. Introduction

1.1. Etiological factors for viral encephalitis

Encephalitis is defined as the presence of an inflammatory process of the brain in association with clinical manifestation of neurological system of the individual. In other words, onset of central nervous system (CNS) symptoms due to infections of the brain. Described pathogens reported as to be the causative agents for encephalitis, the majority of them are viral in origin, but sometimes bacteria or fungi or a postinfectious process. Inspite of the fact that molecular biology researches advance, new era of essentials elements in diagnosis commences, extensive tests are being used widely, the etiology of encephalitis remains unclear and unknown in a considerable degree of the patients [1-3].

Acute encephalitis includes a medical emergency. In most cases, the presence of focal neurological signs and mostly focal seizures will distinguish an encephalitic situation from an encephalopathic process. The diagnosis of encephalitis is suspected in a febrile patient who comes with altered conciousness and signs of cerebral dysfunction. The latter are so wise, therefore the dilemma of diagnosis starts with the beginning, and continues with the determination of the relevance of an infective agent. These agents may play a role in the neurologic manifestations of illness, but not necessarily by directly invading the CNS. Apart from this, there is a big challenge in distinguishing between infectious encephalitis and posinfecitious encephalomyelitis. Vaccination programs were completed in the Western world already; therefore postinfectious or posimmunizative type encephalitis or encephalomyelitis (mainly acute disseminated encephalomyelitis [ADEM]) should be different in etiological aspect, since ADEM is mediated by an immunologic response to antigenic stimuli from infecting microorganisms or immunization. Noninfectious CNS diseases (e.g., fibroelastic tissue diseases, vasculitis, collagenous diseases, and paraneoplastic syndromes) can mimic encephalitis, or present with similar
outcomes to those of encephalitis and should be accounted in the differential diagnosis. Herpes simplex encephalitis (HSE) is the commonest sporadic acute viral encephalitis in developed countries. The emergence of unusual forms of zoonotic encephalitis have an important public health problem all over the world. Vaccination and vector control measures are useful preventive strategies in the management of certain arboviral and zoonotic encephalitis [4].

Since the medical situation is emergent, in the approach to the patient with encephalitis, the main attempt should be carried out to build a reliable etiological diagnosis. Although, there are no definitive effective treatment – with few exceptions, no specific therapy is available for most forms of viral encephalitis – in many cases, identification of a specific agent – if possible – may be important for prognosis, potential prophylaxis, counseling of patients and family members, and public health issues [1].

Epidemiological clues that may help in directing the investigations for an etiologic diagnosis include season, geographical localization, travel history, occupational status, insect and animal contact, vaccinations, immunization of the insult. Therefore clinic approach should be carried out for etiology. Possible etiological agents of encephalitis – mainly viral – based on epidemiology and related risk factors are represented in Table 1. This table is revised from Infectious Diseases Society of America (IDAS) Guidelines 2008:

| Epidemiology or risk factors        | Possible infectious agent(s) for encephalitis |
|------------------------------------|----------------------------------------------|
| Agammaglobulinemia                 | Enterovirus                                  |
| **Age**                            |                                              |
| Neonates                           | Herpes simplex virus (HSV) type 2, Cytomegalovirus (CMV), Rubella virus, |
| Infant and children                | Eastern equine encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, Influenza virus, La crosse virus |
| Elderly persons                    | Eastern equine encephalitis virus, St Louis encephalitis virus, West Nile virus, sporadic Creutzfeldt–Jacob disease (sCJD) |
| **Animal contacts**                |                                              |
| Bats                               | Rabies virus, Nipah virus                    |
| Birds                              | West Nile virus, Eastern equine encephalitis virus, |
| Cats                               | Japanese virus,                             |
| Dogs                               | Rabies virus,                               |
| Horses                             | Rabies virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Hendra virus |
| Skunks                             | Rabies virus,                               |
| Swine                              | Japanese encephalitis virus, Nipah virus     |
| Immunocompromised persons          | Varicella zoster virus (VZV), CMV, Human herpesvirus 6, West Nile virus, HIV, JC virus |
| Unpasteurized milk                 | Tick-born encephalitis virus,               |
| Insect contact                     |                                              |
**Epidemiology or risk factors** | **Possible infectious agent(s) for encephalitis**
--- | ---
**Mosquitoes** | Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis virus, St Louis encephalitis virus, Murray Valley encephalitis virus, Japanese encephalitis virus, West Nile virus
**Ticks** | Tick-borne encephalitis virus, Powassan virus,
**Occupation** | 
**Exposure to animals** | Rabies virus,
**Exposure to horse** | Hendra virus,
**Exposure to old World primates** | B virus
**Laboratory workers** | West Nile virus, HIV,
**Physicians and health care workers** | VZV, HIV, Influenza virus, measles virus,
**Veterinarians** | Rabies virus,
**Person to person transmission** | HSV (neonatal), VZV, Venezuelan equine encephalitis virus (rare), Poliovirus, nonpolio Enterovirus, Measles virus, Nipah virus, Mumps virus, Rubella virus, Epstein-Barr virus (EBV), Human herpesvirus 6, B virus, West Nile virus (transfusion, transplantation, breast feeding), HIV, Rabies virus (transplantation), Influenza virus,
**Recent vaccination** | Acute disseminated encephalomyelitis,
**Recreational activities** | 
**Camping/hunting** | All agents transmitted by mosquitoes and ticks (see above)
**Sexual contact** | HIV,
**Spelunking** | Rabies virus,
**Swimming** | Enterovirus,
**Seasons** | 
**Late summer/early fall** | All agents transmitted by mosquitoes and ticks (see above), Enterovirus
**Winter** | Influenza virus
**Travel** | 
**Africa** | Rabies virus, West Nile virus,
**Australia** | Murray Valley encephalitis virus, Japanese encephalitis virus, Hendra virus
**Central America** | Rabies virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis virus, St Louis encephalitis virus,
**Europe** | West Nile virus, Tick-borne encephalitis virus,
**India, Nepal** | Rabies virus, Japanese encephalitis virus,
**Middle East** | West Nile virus
**Russia** | Tick-borne encephalitis virus,
**South America** | Rabies virus, Eastern equine encephalitis virus, Western equine encephalitis virus, St Louis encephalitis virus,
**Southeast Asia, China, Pasific Rim** | Japanese encephalitis virus, Tick-borne encephalitis virus, Nipah virus
**Unvaccinated status** | VZV, Japanese encephalitis virus, Poliovirus, Measles virus, Mumps virus, Rubella virus

**Table 1.** Possible Etiology of Viral Encephalitis [1]
Clinical findings (physical and specific neurological signs and symptoms) may indicate certain causative agents in patients with encephalitis (Table 2). This table is again revisely taken from the same guideline mentioned in the previous paragraph [1];

| Clinical presentation | Possible infectious agent |
|-----------------------|---------------------------|
| General findings      |                           |
| Lymphadenopathy       | HIV, EBV, CMV, Measles virus, Rubella virus, West Nile virus, |
| Parotitis             | Mumps virus               |
| Rash                  | VZV, B virus, Human herpesvirus 6, West Nile virus, Rubella virus, certain Enteroviruses, |
| Respiratory tract findings | Venezuela equine encephalitis virus, Nipah virus, Hendra virus, Influenza virus, Adenovirus, |
| Retinitis             | CMV, West Nile virus,     |
| Urinary symptoms      | St Louis encephalitis virus (early) |
| Neurological findings |                           |
| Cerebellar ataxia     | VZV (in children), EPV, Mumps virus, St. Louis encephalitis virus, |
| Cranial nerve abnormalities | HSV, EBV, |
| Dementia              | HIV, Human transmissible spongiform encephalopathies, sCJD and variant Creutzfeldt-Jacob disease (vCJD), Measles virus (Subacute sclerosing panencephalitis (SSPE)) |
| Parkinsonism          | Japanese encephalitis virus, St. Louis encephalitis virus, West Nile virus, Nipah virus, |
| Poliomyelitis-like flaccid paralysis | Japanese encephalitis virus, West Nile virus, Tick-born encephalitis virus, Enterovirus (enterovirus-71, coxsackieviruses), Poliovirus |
| Rhombencephalitis     | HSV, West Nile virus, Enterovirus 71 |

Table 2. Possible etiological agents of viral Encephalitis based on clinical findings

2. CSF findings in viral encephalitis

Cerebrospinal fluid (CSF) is produced in choroid plexus of brain ventricules and in subarachnoid pial surface. Noninfective CSF contains maximum 5 weight blood cells (WBC) in a mm$^3$. The protein content in normal CSF does not exceed 50mg/dl and CSF glucose is 50-70 % of serum glucose levels. Central nervous system infections alter this normal content in varied degrees. Thus, knowing these alterations in various infectious and noninfectious situations is crucial for attaining veritable diagnosis. CNS infections should be born in mind in patients, who attain to emergency departments with fever, impaired consciousness and findings attributed to nervous system. Obtaining CSF with lumber puncture performed in
early period leads to at once, differentiation of central pathologies from systemic ones, of infectious etiologies from noninfectious causes, and getting data concerning the character of a possible central nervous system infection; therefore CSF analysis maintains its importance as a valid method currently, for searching brain infections.

Lumbar puncture is performed generally from L4-5 intervertebral space. However L3-4 and L5-S1 intervertebral spaces are also utilized. Sufficient CSF sample should be obtained for routine laboratory tests, and a certain amount should be spared for advanced tests. Initially, protein and glucose levels are analysed from obtained sample, white blood cell count is done, and cultural analyses are performed. Opening pressure and protein concentration are increased, and glucose levels are decreased in bacterial meningitis. Polymorphonuclear cells (PNL) are usually found. Opening pressure is normal or mildly increased however in viral encephalitis and meningitis. In a classical viral encephalitis glucose levels are normal, but protein concentration is found to be mildly or moderately increased. CSF findings in several infectious situations is summarized in Table 3.

|                  | Bacterial meningitis | Viral encephalitis | Fungal meningitis | Tuberculose meningitis |
|------------------|----------------------|--------------------|------------------|------------------------|
| Pressure         | Increased            | Normal-mildly increased | Normal-mildly increased | Increased             |
| Glucose          | Low                  | Normal             | Low              | Low                    |
| Cell count       | PNL                  | Mononuclear        | Mononuclear      | PNL/mononuclear        |
| Protein          | High                 | High               | High             | High                   |

Table 3. General characteristics of various CNS infections

In viral encephalitis, a more important problem is to find out the etiological agent and to apply therefore the appropriate antiviral agent beginning from the early period of the disease. Nevertheless, CSF findings, as they are analysed by routine tests, are not specific in viral encephalitis, and couldn’t be helpful to distinguish different etiological agents. These findings combined with radiological data could also not be assistant, and determination of etiology may be delayed. As a matter of fact, various serological methods, cell cultures and genom analyses are widely utilised currently. Methods to apply should be adapted to geographical factors, to epidemological data, and to travel history in a specific individual. Negative results does not always rule out a certain agent, therefore repeated tests could be needed.

A hemorrhagic CSF could be seen in Herpes simplex type I encephalitis [5]. Lymphositic pleocytosis (10-500 mononuclear cell/mm³) and increased protein concentrations are usually found [6]. However, in immunocompromised patients especially, one could not encounter typical pleocytosis. Thus, CSF findings could be misleading in such situations; before ruling out the disease or an etiological agent, a wider CSF screen is needed in these patients. Determination of HSV-DNA with polymerase chain reaction (PCR) is a widely utilised method today. As a gold diagnostic standart currently, PCR’s sensitivity is 95 % in Herpes simplex...
type I, and its specificity is 100% [7]. Since the identification of HSV-I in the early period of the disease is an ongoing problem, the test should be repeated after 3-7 days in cases with negative results [1]. Studies searching for the association between HSV-DNA load and disease prognosis haven’t revealed consistent results hitherto; hence, further studies are needed [8]. Isolation of the virus in cell culture is also possible, but methods sensitivity is quite low and is not invoked in clinical practice widely. Another method is to determine specific antibodies. Blood/CSF antibody ratio below 20/1 exposes the intrathecal synthesis and is useful in diagnosis of Herpes simplex encephalitis in a considerable degree. Positive PCR results are tend to diminish with the parenteral application of acyclovir, possibility of a positive test after second week is quite decreased; in contrast, in this period of the disease, specific antibodies are easily determined. The fact that patients with negative PCR and positive oligoclonal bands are frequently encountered in a specific period of the disease suggests that these two methods are sensitive to different stages of the diseases [9]. Recent studies displayed some inflammatory cytokine level alterations in CSF. While in the early period of the disease the IFN-γ and IL-6 levels are high, at the period of 2-6 weeks, TNF-α, IL-2 and soluble CD8 levels are found to be increased [10]. Maybe, these findings are reflecting the neuronal damage and inflammatory reaction, however the clinical importance of them are not well established currently.

A lymphocytic pleocytosis is seen in Varicella zoster virus (VZV) encephalitis (below 100 cells/mm$^3$), and increased protein concentrations and normal glucose levels are found. Opening pressure maybe increased [6]. In cell culture, the virus is rarely isolated. VZV-PCR is a useful technique for determining the agent. Once again, negative results do not rule out the virus. In many cases, virus DNA is diminished in CSF after the first week, hence the way to be chosen is to analyze intrathecal antibodies at this period. Determining the ratio of IgG antibodies to blood content or IgM levels are useful. VZV glycoprotein E does not express antigenic resemblance with the herpes simplex virus, and is easily determined with performing ELISA. This method has a high specificity and sensitivity for VZV encephalitis, it may also be utilised for the differential diagnosis with herpes virus [11]. The test to be chosen in Ebstein-Barr virus and in Citomegalovirus encephalitis is again PCR. Negative results do not exclude the agents. Determining the alterations of IgM and IgG levels with serological analyses maybe useful in EBV encephalitis. HHV-6 and HHV-7 PCR tests should be added to routine CSF screen in immunocompromised patients [5]. It should also be noted that HHV-6 PCR does not distinguish latent infection from active encephalitis. Diagnostic methods for encephalitis caused by herpesvirus family is shown in Table 4.

Besides HSV and VZV, PCR test is trusty also in JC virus. In immunocompromised patients in whom multifocal leucoencephalopathy is suspected, PCR technique is highly specific. Pleocytosis is characteristic in Mumps encephalitis. Interestingly however, protein levels are generally normal and glucose concentrations are decreased. The disease should be differentiated from Lymphocytic choriomeningitis virus, since decreased glucose levels are resulted also from that agent caused encephalitis (Figure 1). Cell culture and PCR are equally useful. Specific antibodies should be investigated if PCR is negative. Four fold increase in IgG levels or determining IgM are helpfull, but it should be born in mind that Mumps spe-
cific antibodies may express cross-reaction with Parainfluenza virus antibodies [6]. In the course of encephalitis caused by Enteroviruses, CSF cell count is generally normal, or a mildly mononuclear pleocytosis is present. Glucose levels are normal and protein concentration is increased. Method to be chosen is RT-PCR. Sensitivity and specificity are 86 % and 100 % respectively. Cell culture may also be helpfull. Despite Influenza encephalitis is rarely reported, it should be investigated in pandemic situations and/or in conditions, in which no other etiological agent is determined. Routine CSF screen is usually normal. The etiological analysis is performed by RT-PCR and cell culture in suspected cases.

| Virus      | Method                          |
|------------|---------------------------------|
| HSV-1      | PCR, quantitative PCR, routine serology |
| HSV-2      | PCR, routine serology, culture   |
| EBV        | PCR, routine serology           |
| CMV        | PCR                             |
| HHV-6, HHV-7 | PCR, routine serology, culture                          |
| VZV        | PCR, routine serology, VZV Ge   |

Table 4. Diagnostic methods in herpesviridae family.

In encephalitis caused by Flaviviruses, clinical suspicion maintains its importance. Methods that target Flaviviruses should be added to routine CSF analyses in endemic regions, or in patients who have a travel history; at times, repeated lumbar punctures are needed for determining the etiological agent. In West nile virus encephalitis, domination of polymorphonuclear leukocytes in hyperacute period, leaves its place to lymocytes afterwards. CSF
protein concentrations are usually increased, glucose levels are normal. RT-PCR is assistant, but it is not possible in late stages of the disease to capture the virus RNA [12]. Virus isolation by means of CSF cultures is also utilised [13]. Today the most valid methods are serological approaches. The success of ELISA in detecting WNV-specific antibodies is increased in 8-21 days after the beginning of clinical symptoms. Similar serological methods can be used in other Flavivirus infections. In Japan encephalitis, for example, the valid method currently is ELISA capture of JE-IgM [14] (Table 5). Various biomarkers, which are detected in CSF in the course of WNV encephalitis may reflect the severity of disease and neuronal damage. In 58% of cases with WNV, NfH-SM135 and GFAP-SM126 can be found positive, S100B positivity is seen in 90% of this same group [15]. In Eastern equine encephalitis, leucocyte count is much more increased, and it can reach 1000-2000 cells per mm³; it should also be noted that dominant cells are polymorphnuclear. CSF findings emerged from various encephalitic situations are summarized in Table 6.

As we mentioned above, a part of recent studies targets on inflammatory responses in CSF. Without question, these biomarkers are not etiology specific. However, they can be used for manifesting the severity of neuroinvasif disease. One of those markers is macrophage migration inhibitory factor (MIF) that increases in CSF in CNS infections [16]. Studies that investigate the association of these factors with possible etiological agents and disease severity is needed (Figure 2).

![CNS INFECTIONS](https://example.com/cns-infections.png)

**Figure 2.** Several biomarkers elevating in CSF during the course of central nervous system infections.

| FLAVIVIRUS INFECTIONS |
|------------------------|
| Elevated protein, high cell count (initially neutrophilic; mononuclear pleocytosis after a certain time), normal glucose concentrations |
| RT-PCR, IgM ELISA capture |
| Virus isolation |

**Table 5.** CSF characteristics and diagnostic methods in flavivirus encephalitides
CSF findings in all types of encephalitis may expose time dependent alterations. In cases with negative PCR, repeated lumbar punctures should be performed, differences in cell count should be observed, PCR studies should be repeated and new cell cultures should be made for virus isolation. This approach is valid in patients receiving empirical antiviral treatment also. For example, it is known that PCR becomes positive several days after the onset of clinical symptoms in herpes encephalitis. PCR test becomes negative after a certain time in HSV and VZV encephalitis. This duration is shorter in patients receiving antiviral therapy. Once again, in herpes encephalitis, intrathecal antibody production commences beginning from the second week. In West Nile virus encephalitis, initial neutrophilic dominance gives way to a lymphocytic pleocytosis. Capturing specific IgM antibodies in the first week after symptom onset leads frequently to negative results. But the chance of detection increases in the following days. Therefore it is crucial to repeat lumbar puncture in such cases. On the other hand, WNV RT-PCR is positive in a narrow period, but the possibility of a positive result decreases as the disease progresses (Table 7).

|                     | cell count | Protein | Glucose            |
|---------------------|------------|---------|--------------------|
| HSV                 | MN         | High    | Normal-Low         |
| VZV                 | MN         | High    | Normal-Low         |
| CMV                 | MN         | High    | Normal             |
| MUMPS               | MN         | Normal-High | Normal-Low     |
| Enteroviruses       | Normal-MN  | High    | Normal             |
| WNV                 | PNL-MN     | High    | Normal             |
| Influenza           | Normal     | Normal  | Normal             |
| JC virus            | Normal     | High    | Normal             |

**Table 6.** CSF characteristics of encephalitis in various viruses.

|                | Cell count   | PCR    | Antibody production |
|----------------|--------------|--------|---------------------|
| HSV<3 days     | Normal-monoruclear | Negative | Negative |
| 3-14 days      | Mononuclear  | Positive | Negative-Positive |
| "/>14 days     | Mononuclear  | Negative-Positive | Positive-Negative |
| WNV <2 days    | Polymorphnuclear | Positive | Negative |
| 2-7 days       | Mononuclear  | Positive-Negative | Negative |
| "/>7 days      | Mononuclear  | Negative | Positive |

**Table 7.** Time dependent alterations of CSF findings in Herpes and WNV.
3. Future prospects of CSF studies for viral encephalitis

Current diagnostic methods which have been described above have been providing valuable proves for diagnostic process of the viral encephalitis but new approaches are needed with increased knowledge of pathogenesis of viral encephalitis. These are must be combined according to clinical picture and possible etiological agents. These promising methods are;

1. Detection of viral genomic materials
   a. RT-PCR, IgM ELISA capture
   b. Detection of viruses
   c. Differentiation of lytic and lstent viral infectivity

2. Evaluation of inflammatory markers
   a. IFN-γ, TNF-α, IL-2, IL-6, CD8
   b. Macrophage migration inhibitory factor (MIF)
   c. Determination of the antibodies

3. Evaluation of tissue and neuronal damage products
   a. NfH-SM135, GFAP-SM126
   b. S100B

4. Prognostic use of CSF findings in viral encephalitis [1, 3, 7].

Related to future prospects of diagnostic methods which will evaluate biomarkers in CSF must be improved as diagnostic and also prognostic methods.

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