Chapter 2

Cerebrospinal Fluid Abnormalities in Viral Encephalitis

Hakan Ekmekci, Fahrettin Ege and Serefnur Ozturk

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54590

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 latters 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 posinfectious 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 account in the differential diagnosis. Herpes simplex encephalitis (HSE) is the commonest sporadic acute viral encehalitis 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 avaliable for most forms of viral encephalitis – in many cases, identification of a spesific 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-born encephalitis virus, Powassan virus,
Occupation	
Exposure to animals	Rabies virus,
Expoure 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-born encephalitis virus,
India, Nepal	Rabies virus, Japanese encephalitis virus,
Middle East	West Nile virus
Russia	Tick-born 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-born 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 Urinary symptoms	CMV, West Nile virus, 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 wight 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 alerations 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 menengitis. Polymorphonuclear cells (PNL) are usually found. Opening pressure is normal or mildly increased however in viral encephalitis and menengitis. In a classical viral encephalitis glucose levels are normal, but protein concentration is found to be mildly or moderatly increased. CSF findings in several infectious situations is summarized in Table 3.

	Bacterial menengitis	**Viral encephalitis**	**Fungal menengitis**	**Tuberculose menengitis**
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 heplfull 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 epidemyological 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 hemorhagic CSF could be seen in Herpes simplex type I encephalitis [5]. Lymphositic pleocytosis (10-500 mononuclear cell/mm^3) and increased protein concentrations are usually found [6]. However, in immuncompromised 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 specifity 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 intratecal synthesis and is usefull 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 inflamatory 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 soluable CD8 levels are found to be increased [10]. Maybe, these findings are reflecting the neuronal damage and inflamatory 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 usefull 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 helpfull. 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 specifity and sensitivity for VZV encephalitis, it may also be utilised for the differential diagnosis with herpes virus [11]. The test to be choosen 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 usefull in EBV encephalitis. HHV-6 and HHV-7 PCR tests should be added to routine CSF screen in immuncompromised 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 herpesviridea family is shown in Table 4.

Besides HSV and VZV, PCR test is trusty also in JC virus. In immuncompromised patients in whom multifocal leucoencephalopathy is suspected, PCR technique is highly specific. Pleocytosis is charactheristic in Mumps encephalitis. Interestingly however, protein levels are generally normal and glucose concentrations are decreased. The disease should be differentiated from Lymphocytic choriomenengit virus, since decreased glucose levels are resulted also from that agent caused encephalitis (Figure 1). Cell culture and PCR are equally helpfull. 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 choosen is RT-PCR. Sensitivity and specifity 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.

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.

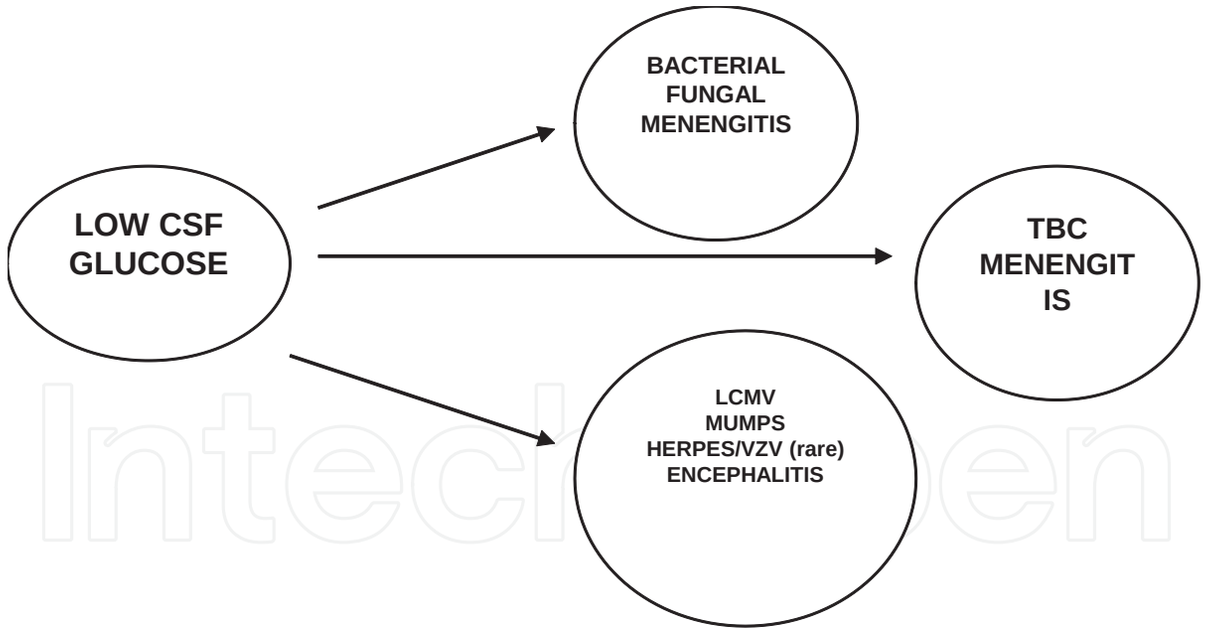

Figure 1. CNS infections, in which low CSF glucose levels are found.

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 lympocytes 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 mm^3; 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).

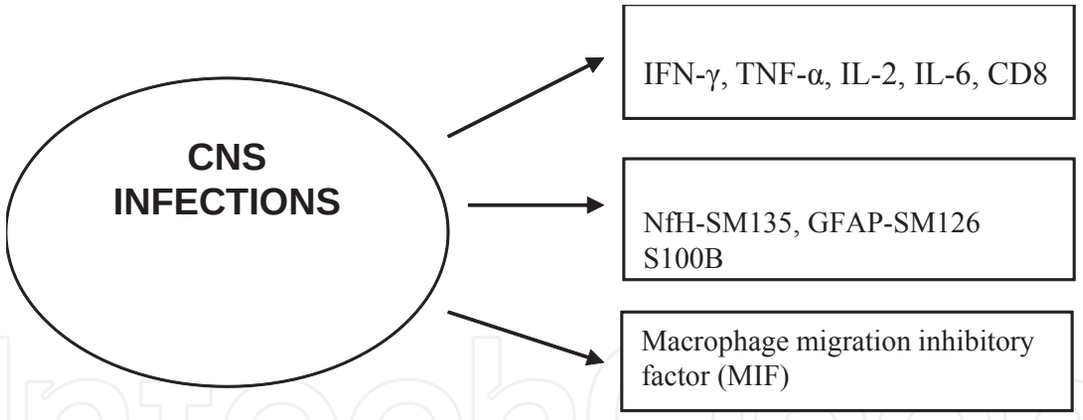

Figure 2. Several biomarkers elevating in CSF during the course of central nervous system infections.

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 aproach is valid in patients receiving amprical 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 neutrophylic dominance gives way to a lymphocytic pleocytosis. Capturing specific IgM antibodies in the first week after syptom 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-mononuclear	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.

Author details

Hakan Ekmekci[1], Fahrettin Ege[2] and Serefnur Ozturk[1*]

*Address all correspondence to: serefnur.ozturk@noroloji.org.tr

1 Selcuk University, Selcuklu Medical Faculty, Department of Neurology, Konya, Turkey

2 Ufuk University, Medical Faculty, Department of Neurology, Ankara, Turkey

References

[1] Tunkel Ar, Glasser CA, Bloch KC, Sejvar JJ, Marra CM, Roos KL, Hartman BJ, Kaplan SL, Scheld WM, Whitley Rj, Infectious disease society of America. The management

of encephalitis: clinical practice guidelines by the infectious diseases society of America, Clin Infect Dis 47 (2008) 303-327

[2] Rantalaiho T, Farkkila M, Vaheri A, Koskinemiemi M. Acute encephalitis from 1967 to 1991. Journal of the Neurological Sciences 2001; 184: 169 – 177.

[3] Steiner I, Schmutzhard E, Sellner J, Chaudhuri A, Kennedy PG. EFNS-ENS guidelines for the use of PCR technology for the diagnosis of infections of the nervous system. Eur J Neurol. 2012 Aug 6. doi: 10.1111/j.

[4] Chaudhuri A, Kennedy PGE. Diagnosis and treatment of viral encephalitis. Journal of postgraduate Medicine 2002; 78: 575 – 583.

[5] Ziai WC, Lewin III JJ, Update in the diagnosis and management of central nervous system infections, Neurol Clin 26 (2008) 427-468

[6] Romero JR, Newland JG, Diagnosis of viral encephalitides: Nonzoonotic-associated viruses, The pediatric infectious disease journal, 25 (2006) 739-740

[7] Jakob NJ, Lenhard TL, Schnitzler P, Rohde S, Ringleb PA, Steiner T, Wildemann B, Herpes simplex virus encephalitis despite normal cell count in the cerebrospinal fluid, Crit care med 40 (2012)1304-1308)

[8] Ziyaeyan M, Alborzi A, Haghighi AB, Moeini M, Pourabbas B, Diagnosis and quantitative detection of HSV DNA in samples from patients with suspected herpes simplex encephalitis, Braz J Infect Dis 15(3) (2011), 211-214

[9] Ambrose HE, Granerod J, Clewley JP, Davies NWS, Keir G, Cunningham R, Zuckerman M, Mutton KJ, Ward KN, Ijaz S, Crowcroft NS, Brown DWG, on behalf of the UK Aetiology of encephalitis study group, Diagnostic strategy to establishetiologies of encephalitis in a prospective cohort of patients in england, Journal of clinical microbiology 49 (2011) 3576-3583.

[10] Kamei S, Taira N, Ishikara M, Sekizawa T, Morita A, Miki K, Shiota H, Kanno A, Suzuki Y, Mizutani T, Itoyama Y, Morishima T, Hirayanagi K, Prognostic value of cerebrospinal fluid cytokine changes in herpes simplex virus encephalitis, Cytokine 46 (2009) 187-193

[11] Grahn A, Studahl M, Nilsson S, Thomsson E, Backström M, Bergström T, Varicella-zoster virus (VZV) glycoprotein E is a serological antigen for detection of intrathecal antibodies to VZV in central nervous system infections, without cross-reaction to herpes simplex virus 1, Clinical and Vaccine immunology, 18 (2011) 1336-1342

[12] LaSala PR, Holbrook M, Tick-Borne Flaviviruses, Clin Lab Med 30 (2010) 221-235

[13] Rossi SL, Ross TM, Evans JD, West nile virus, Clin Lab Med 30 (2010) 47-65

[14] Misra UK, Kalita J, Overview: Japanese encephalitis, Progress in Neurobiology 91 (2010) 108-120

[15] Petzold A, Groves M, Leis AA, Scaravilli F, Stokic DS, Neuronal and glial cerebrospinal fluid protein biomarkers are elevated after west nile virus infection, Muscle Nerve 41 (2010) 42-49

[16] Ostegaard C, Benfield T, Macrophage migration inhibitory factor in cerebrospinal fluid from patients wit central nervous system infection, Critical care 13 (2009)

[17] Crawford JR. Advances in pediatric neurovirology. Curr Neurol Neurosci Rep. 2010 Mar;10(2):147-54