Viral Encephalitis: Etiology, Clinical Features, Diagnosis and Management

Sergio Ferrari[1], Antonio Toniolo[2], Salvatore Monaco[1], Filippo Luciani[3], Francesca Cainelli[4], Andreina Baj[2], Zelalem Temesgen[5] and Sandro Vento[*,3]

[1]*Department of Neurological and Visual Sciences, Section of Neurology, University of Verona, Verona, Italy*

[2]*Laboratory of Medical Microbiology, University of Insubria, Varese, Italy*

[3]*Infectious Diseases Unit, Annunziata Hospital, Cosenza, Italy*

[4]*Department of Emergency Medicine, Annunziata Hospital, Cosenza, Italy*

[5]*Division of Infectious Diseases, Mayo Clinic College of Medicine, Rochester, MN, USA*

Abstract: Viral encephalitis is worldwide spread pathology with high morbidity and mortality. Its incidence is higher in children. Enteroviruses, varicella zoster virus and herpes simplex viruses are the most frequent agents. However, in spite of the use of modern microbiological and radiological methods, an etiological diagnosis is reached in less than 50% of cases, making a careful differential diagnosis with non viral brain diseases imperative. Pathogenesis is elusive and therapy continues to remain supportive in almost all cases, as the only virus-directed treatment is available for herpesvirus-related encephalitis and a role for steroids continues to be debated. Novel and more targeted therapies are eagerly needed.

INTRODUCTION

Encephalitis is an inflammation of the brain parenchyma usually caused by bacteria or viruses, and often associated with meningitis. Viruses vary widely in their potential to produce central nervous system (CNS) infection: for example, rabies virus inevitably and exclusively causes CNS disease, whereas encephalitis is a less common manifestation of infections caused by herpes simplex or varicella zoster viruses. The spectrum of brain involvement and the outcome of the disease are dependent not only on the specific pathogen, but also on the immunological state of the host and on a range of environmental factors. We will focus this review on the most relevant agents of clinically important viral encephalitis: herpes simplex virus 1 (HSV-1), varicella zoster virus (VZV), enteroviruses, Epstein-Barr virus (EBV), Tick borne (TBE), human herpesvirus 6 (HHV-6), measles virus (MV, the agent of subacute sclerosing panencephalitis, SSPE), rabies, West Nile Virus (WNV), Human immunodeficiency virus (HIV). We will not consider more recently described viruses, such as Nipah or Japanese encephalitis viruses, as these have been thoroughly examined in recent reviews. Tables **1a-c** list the main agents of viral encephalitidis.

Epidemiology

Viral encephalitis is of public health concern worldwide because of its high morbidity and mortality as well as considerable economic costs. Incidence varies between studies but is generally between 3.5 and 7.4 per 100,000 patient-years [1], and is higher in children. Although both genders are affected, most studies have shown a slight predominance in males. Unfortunately, few population-based studies have been done, the disease is underreported, and in many cases the cause remains obscure. Data on the incidence of different etiological agents are contradictory. For example, a collaborative study done in Finland using gene amplification to detect various viruses in CSF samples of 3231 patients with encephalitis, meningitis, and myelitis, reported VZV as the most frequently involved agent (29% of cases). HSV and enteroviruses accounted for 11% of cases each and influenza A virus for 7% of cases [2]. In contrast, in a recent report from Turku (Finland) of 144 consecutive adults with encephalitis or aseptic meningitis, viral etiology was recognised in 72 patients; 46% of these cases were attributed to enteroviruses, 31% to HSV-2, 11% to VZV, and 4% to HSV-1 [3]. The difference may be partly explained by the fact that in the latter paper, the authors examined only immunocompetent adults, patients with meningitis and encephalitis were analysed separately, and the 5-year study period was long enough to reduce effects of epidemics and seasonal variation. HSV-1/2 cause the most severe forms of infections of the human brain. Currently, herpes simplex encephalitis (HSE) is estimated to occur in approximately 1 in 250,000 to 1 in 500,000 individuals per year [4]. In the US the estimated incidence is approximately 1 in 300,000 individuals [4], similar to those in England [5] and Sweden [6]. HSE occurs throughout the year and in patients of all ages. Around one-third of cases occurs between 6 months and 20 years of age; approximately half of the patients are older than 50 years [7]. Both genders are equally affected.

Pathogenesis and Histopathology

Viruses access the CNS by either the neuronal or haematogenous route [8-10]. The latter is most common and in arthropod-borne infections is associated with alterations of the blood-brain barrier. In TBE transient viraemia follows viral replication in the skin after an insect bite, with consequent

*Address correspondence to this author at the Infectious Diseases Unit, Annunziata Hospital, 87100 Cosenza, Italy; Tel/Fax: 39.0984.681360;
E-mail: ventosandro@yahoo.it

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Table 1. Selected Etiologic Agents of Viral Encephalitis

(a) DNA Viruses

Herpesviridae	Herpes Simplex Virus (HSV)
	Varicella- Zoster Virus (VZV)
	Epstein-Barr Virus (EBV)
	Cytomegalovirus (CMV)
	Human Herpes Virus-6 (HHV-6)
Polyomaviridae	JCV
	BKV
Adenoviridae	Adenovirus

(b) RNA Viruses

Picornaviridae	Enteroviruses Poliovirus
Retroviridae	Human Immunodeficiency Virus-1/2 (HIV-1/2)
Rabdhoviridae	Rabies
Paramyxoviridae	Mumps
	Measles

(c) Arbovirus Group

Togaviridae	Eastern Equine Encephalitis (EEE)	
	Western Equine Encephalitis (WEE)	
	Venezuelan Equine Encephalitis (VEE)	
Flaviviridae	St Louis Encephalitis (SLE)	
	West Nile Virus (WNV)	
	Japanese Encephalitis (JE)	
	Powassan Virus (POW)	
	Murray Valley Encephalitis (MVE)	
	Tick-Borne Encephalitis Viruses (TBEV)	Far Eastern Subtype (FE-TBEV)
		Siberian Subtype (S-TBEV)
		Western Subtype (W-TBEV)
Bunyaviridae	California Group	California Virus
		La Crosse Virus (LAC)
	Sandfly Fever Group	Toscana Virus (TOSV)

invasion of the reticuloendothelial system. Secondary viraemia leads to infection of other organs, including the CNS. In acute viral encephalitis, a remarkable pathological finding is the infiltration of mononuclear inflammatory cells in the Wirchow-Robin spaces and in the meninges around the wall of vessels (perivascular cuffing). With further disease progression, astrocytosis proliferation and hypertrophy of microglial cells with formation of microglial aggregates (microglial nodules) and neuronophagia (clusters of microglial cells surrounding a dead neuron) become prominent histopathological findings. The majority of inclusions are intranuclear but the only pathognomonic one is the intracytoplasmic Negri body in rabies. The pathologic changes induced by replicating HSV include ballooning of infected cells and intranuclear eosinophilic amorphous or droplet-like bodies surrounded by a clear halo, with chromatin margination at the nuclear membrane (Cowdry type A inclusions). Intranuclear type A inclusions are similar in HSV, VZV and CMV; a better identification of the viral particles is obtained through electron microscopy, immunohistochemical and *in situ* hybridisation techniques. An influx of mononuclear cells in infected tissue ensues. HSE is associated with acute inflammation, congestion, and/or haemorrhage, most prominently in temporal lobes and usually asymmetrically in adults. Adjacent limbic areas are also involved, and the meninges overlying temporal lobes may be congested. Approximately two weeks later, frank necrosis of the involved brain areas occur.

Access to CNS by the intraneuronal route occurs typically in rabies (limbic system), and in HSV infections. Once the virus has reached the brain, it may remain confined to a few cells or spread to adjacent tissues either by cell-to-cell transmission or through extracellular spaces; afterwards, HSV may remain in a latent state within the CNS [11, 12].

Though largely investigated, the pathogenesis of HSE in children older than 3 months, adolescents and adults remains somewhat obscure. Both primary (in around one-third of the cases, generally younger than 18 years of age) and recurrent HSV infections can cause encephalitis. Of the two-thirds of cases occurring in the presence of pre-existing antibodies, only 10% of patients have a history of recurrent herpes labialis. Patients with pre-existing antibodies are thought to have HSE as a consequence of HSV reactivation [13]. Interestingly, when the genomic DNA from peripheral (labial) and CNS isolates are compared by restriction analysis, usually identical, but also different isolates are recovered [14]. The access of HSV to CNS in primary infection is still debated, with both the olfactory and trigeminal nerves proposed as prevailing routes. In patients with HSE, HSV particles have been demonstrated by electron microscopy along the olfactory tract in some individuals [15, 16]. Animal models support the view that the olfactory tract provides an entry route into the CNS thus causing localisation of infection in brain regions analogous to the medial temporal structures of humans [17, 18]. Where does reactivation of latent virus occur? Reactivation within the brain tissue has not been demonstrated. Although it has been proposed that this event takes place in the olfactory bulb or the trigeminal ganglion, with subsequent neuronal transmission to the CNS [17], it should be noted that HSE is rarely associated with recurrent herpes labialis. What about host immunity? It is conceivable that the CNS is particularly prone to HSV infection since intraneuronal spread hides the virus from host defence mechanisms. The picture is further complicated by the observation that the HSE prevalence is the same both in normal and immunosuppressed hosts. However, the presentation is atypical in immunosuppressed individuals with a subacute and progressively deteriorating course [19].

Clinical Presentation

Viral encephalitis can present in forms of low or mild severity that heal spontaneously or in much more aggressive forms with a poor prognosis and severe neurological sequelae in survivors. The prodromal signs and symptoms are

those of a classic viral infection: fever and headache, possibly accompanied by lymphadenophathy, nausea or vomiting. After a few days, symptoms of CNS involvement become manifest with altered mental status, considerable irritability and agitation, personality changes; seizures (focal or generalised) may occur, sometimes accompanied by focal neurological signs. Patients may then become lethargic or comatose; death eventually ensues. Stiff-neck is a sign of meningeal involvement.

Fever is one of the most frequent features at presentation, and its absence should cast doubts on diagnosis. Prognosis is poorer in infants younger than 1 year and adults over 55. Young children may have a stormy course for several days because of severe cerebral oedema.

Enterovirus Encephalitis

Enteroviruses seem to account for the majority of cases of viral encephalitis both in children and adults [20]. Infection is seasonal in temperate climates (summer and autumn peaks) but high the year round in tropical and subtropical climates. Although enteroviral encephalitis may be accompanied by mucocutaneous manifestations, including localised vesicles (e.g. hand, foot, and mouth disease), herpangina, and generalised maculopapular rash, these symptoms can be absent. Over the last 10 years, outbreaks of neurovirulent Enterovirus 71 have been reported from Japan, Malaysia, and Taiwan. A high mortality rate (19.3%) has been reported from Taiwan in children below 5 years of age [21].

Unfavourable outcomes (death or neurological sequelae) of enteroviral encephalitis have been associated with younger age (< 4 years), high peak leukocyte counts (> 13,000/mm^3), seizures, skin rash, myoclonic jerks, lower CSF viral yield rate, oral ulcers, enterovirus 71 as etiologic agent [21].

Herpes Simplex Encephalitis (HSE)

In HSE grey matter dysfunction of temporal and frontal lobes is a dominant feature, and the disease presents with personality changes, confusion, and disorientation. Seizures occur in half of the patients, focal neurological signs (hemiparesis) in about a third. Since prognosis is dependent on early initiation of treatment, there is a need for immediate and accurate diagnosis. For this reason, lumbar puncture should be preceded by neuroimaging only when focal neurological signs are present. CSF is abnormal in more than 95% of HSE cases; moderate pleocytosis is found, usually both of mononuclear white blood cells and red blood cells, the latter due to the haemorrhagic nature of the infectious process.

Varicella Zoster Encephalitis

VZV encephalitis occurs mainly in young adults and infants [22]. Seizures occur in 29 to 52% of cases. Focal neurological abnormalities include ataxia, hypertonia or hypotonia, hemiparesis, and positive plantar responses. Mortality varies from 5 to 10% [23]. Intranuclear inclusions and haemorrhagic necrosis, suggestive of varicella encephalitis, have been reported also in immunocompromised patients and in neonates [24].

Epstein Barr virus (EBV) Encephalitis

EBV encephalitis may involve not only temporal lobes but also different sites, including the cortex, brainstem and basal ganglia. Importantly, signs and symptoms of infectious mononucleosis, such as pharyngitis, adenopathy, splenomegaly, and atypical lymphocytosis are often absent [25, 26]. IgM against viral capsid antigens can be undetectable [27]. CSF abnormalities, if present, are subtle, with mild increase in pressure, mild mononuclear pleocytosis (≤ 300 cells/mm^3 in one series) [26] and slightly elevated proteins. Diagnosis is suggested by the occurrence, in more than a third of patients, of movement disorders, such as chorea, and focal disturbances (hemiparesis) [26]. Although EBV encephalitis runs frequently a benign course, neurologic sequelae may occur [26] and the disease can occasionally be fatal [27].

Human Herpesvirus 6 (HHV-6) Encephalitis

HHV-6, the causal agent of exanthema subitum, infects nearly all children by 3 years of age; two variants, A and B, have been identified [28]. Both of them are neurotropic *in vivo* and reside latently in the adult brain, complicating attempts to link HHV-6 to neurological disease [29]. However HHV-6 is considered a possible cause of encephalitis in immunosuppressed children. In patients undergoing allogeneic haematopoietic stem cell transplantation, a form of acute limbic encephalitis (characterised by anterograde amnesia, inappropriate ADH secretion syndrome, mild CSF pleocytosis, temporal EEG abnormalities) has been linked to HHV-6 [30]. Recently, HHV-6-associated rhombencephalitis, presenting with seizures, ataxia, and myoclonus, has been described in immunocompetent children [31]. Rare cases of encephalitis have been attributed to HHV-6 in adults [32-37].

Tick-Borne Encephalitidis

Viruses transmitted to humans by tick-bite are important causes of CNS infections worldwide. TBE is caused by two strictly but biologically distinct flaviviruses. Seasonal outbreaks occur in rural areas of Russia (Russian Spring-Summer Encephalitis, RSSE) and in Central Europe (Central European Encephalitis, CEE); transmission is mediated by the hard ticks *Ixodes persulcatus* and *Ixodes ricinus*, respectively (*I. ovatus* and *I. gibosus* may be additional vectors). The incubation period is 2 to 28 days. The clinical presentation of RSSE and CEE is similar, but RSSE is generally monophasic whereas CEE normally occurs in two stages. Severe cases are more frequent in RSSE. Importantly, around 30% of TBE patients do not recall any tick bite [38]. CEE is heralded by a few days of fever, headache, and muscle pain, followed by an asymptomatic period (2-10 days); later, symptoms and signs of encephalitis ensue. The mortality rate of RSSE is up to 25%, whereas in CEE it does not exceed 4% [39]. Recovery from severe cases is slow, and 20% of patients may have severe neurologic sequelae.

Measles Encephalitis

In MV infection, CNS involvement can occur early or late after acute measles, causing acute and subacute forms of encephalitis. Acute postinfectious measles encephalitis generally occurs in immunocompetent patients (mostly children and adolescents) during the exanthematic phase and within 8 days after clinical onset [40]. The mortality rate is between 10 and 20 %, while sequelae are observed in 20 to 40 % of patients who recover [40]. Onset of encephalitis at a later

phase results either in measles inclusion body encephalitis (MIBE) or in subacute sclerosing panencephalitis (SSPE). MIBE is a subacute/chronic neurologic disease that may be observed in immunosuppressed children and young adults.

SSPE is a demyelinating CNS disease occurring several years (generally 6-8) after acute measles infection, with a prevalence of 1 in 100,000 to 1 in 1 million infected children. The disease has an insidious onset characterised by cognitive deterioration, poor school performance, ataxia, behavioural changes, myoclonus, possibly seizures, and chorioretinitis with partial visual deficit. Although progression differs in time and remissions may occur, death invariably occurs within 1 to 3 years [41]. Mutant MV strains (SSPE virus) can be recovered from the brain tissue of SSPE patients.

Rabies

Rabies is the only human infection causing acute encephalitis with a near-100% mortality rate. The incubation period varies from 5 days to over 6 months, but is usually of 20-60 days [42]. After a prodromal period of malaise, anxiety, pain or itching at the site of bite, and fever, patients develop encephalopathic (hyperactivity and characteristic hydrophobic spasms of the sternomastoid, diaphragm and other inspiratory muscles, typical of the so called 'furious rabies') or paralytic neurological signs. Respiratory and heart failure following hydrophobic spasms are deadly in one-third of cases. Other clinical findings include Cheyne-Stokes respiration, III, IV and IX cranial nerves palsies and signs of the amygdaloid nuclei damage. Intermittent episodes of hallucinations and maniacal behaviour are also observed. Without intensive care, the illness leads to coma, flaccid paralysis and death in few days from onset.

West Nile Virus

West Nile virus, a flavivirus maintained worldwide in an enzootic cycle and transmitted primarily between avian hosts and mosquito (genus *Culex*) vectors, can occasionally infect humans, but most individuals remain asymptomatic. Symptoms may develop in 20-40% of subjects [43, 44] after an incubation period of 2-14 days. The vast majority of symptomatic patients present with flu-like symptoms (West Nile fever), i.e. fever, headache, malaise, myalgia, fatigue, skin rash, lymphadenopathy, vomiting, diarrhoea [45]. Less than 1% of infected individuals develop neuroinvasive diseases, i.e. meningitis, encephalitis, and/or acute flaccid paralysis. Although clinical features of these syndromes may overlap in the same patient, a large study of 228 patients found that most patients can be classified as either having meningitis or encephalitis and that patients with the latter have a higher mortality rate and more severe complications [46].

West Nile encephalitis follows selective neuroinvasion in certain cell populations (substantia nigra in brainstem, basal ganglia [47] and cerebellum) and is characterised by altered consciousness, disorientation, focal neurological signs and symptoms (dysarthria, seizures, tremor, ataxia, involuntary movements, parkinsonism) [46, 47].

Human Immunodeficiency Virus

During primary HIV infection, neurological manifestations, ranging from severe and persistent headache to clinical signs suggestive of encephalitis and/ormeningitis, can occur. Headache and fever are prominent symptoms [48]. HIV-RNA levels are high in cerebrospinal fluid. Although recovery without antiretroviral drugs usually follows, these can be administered to try and speed it.

Patients with chronic HIV infection can also, albeit rarely, develop acute neurologic syndromes while having very elevated cerebrospinal fluid HIV loads. Resolution of symptoms after changes in, or initiation of, antiretroviral therapy occur [49].

Immune-Mediated Post-Infectious Inflammatory Encephalopathies

In addition to acute viral-induced brain damage, immune-mediated inflammatory encephalopathies can be observed days to a few weeks after a viral (rubella, mumps, measles, EBV, VZV, influenza) or bacterial infection, but also following vaccination, especially with smallpox and rabies vaccines.

This condition, named acute disseminated encephalomyelitis (ADEM), must be distinguished from infectious encephalitis and non-infectious encephalopathies. ADEM is typically monophasic, with multifocal symptoms appearing within days, or sequentially over weeks, either in the presence or absence of fever and meningismus [50]. Neurological symptoms (including focal motor and sensory deficits, optic neuritis, cranial neuropathies, seizures, myoclonus, and ataxia) mainly correlate with demyelinating white matter changes at brain MRI, although inflammatory foci affect the cortical and subcortical grey matter. An acute variant of ADEM, named acute hemorrhagic leucoencephalomyelitis, is a rare and usually fatal condition. ADEM and its variants should be differentiated from demyelinating-like, ischemic, and haemorrhagic lesions observed in large- and small-vessel arteritis caused by VZV infection, and, in addition, from other demyelinating diseases, including multiple sclerosis-like syndromes associated with primary HHV-6 infection. In rare cases, transverse myelitis, optic neuritis, and neuromyelitis optica occur as isolated manifestations of ADEM. The range of post-infectious neurological complications is not limited to CNS, but includes also a bulbar variant of acute inflammatory demyelinating neuropathy (AIDP) after VZV infection, and acute brachial plexopathy in patients with antecedent CMV disease [51]. These disorders should be differentiated also from acute flaccid paralysis secondary to flaviviruses of the TBE complex, non-polio enteroviruses, coxsackievirus, and paralytic rabies.

Non-Infectious Diseases Mimicking Viral Encephalitis

A wide spectrum of acute, subacute, and chronic medical conditions involving the CNS present with neurological signs and symptoms that may overlap with those observed in the course of viral encephalitis, myelitis, and encephalomyelitis (Table **2**).

While a detailed discussion of these disorders is beyond the scope of this review, we point to specific clues in clinical presentation, imaging findings and laboratory results that may be of help for differential diagnosis. In approaching a patient with suspected encephalitis it is important to define the neurological syndrome, assess whether the observed functional or structural CNS alterations involve the grey or white matter or both, obtain evidence of inflammation, and

Table 2. Non-Infectious Diseases Mimicking Viral Encephalitis

Condition	Diagnostic Features
Limbic Encephalitis	Anti-Hu, -Ma2, -amphiphysin, -CRMP5/CV2 (all frequently associated with cancer), -VGKC, -NMDA receptor
Hashimoto's Encephalitis	Anti-thyroid peroxidase, anti-thyroid microsomal antibodies
Sjögren Sindrome	Anti-SSA/Ro, -SSB/La antibodies
Systemic Lupus Erythematosus (SLE)	Anti-nuclear, -double-stranded DNA, antiphospholipid antibodies
Acute Disseminate Encephalomyelitis (ADEM)	Predominant demyelinating lesions at brain MRI
Vasculitis of CNS	CNS angiography, ANCA
Bulbar Variant of GBS	CSF albuminocytological dissociation
Primary and Metastatic Brain Tumours	CNS imaging
Metabolic and toxic diseases	Liver and renal function toxicology screening
Epileptic status	EEG recordings

Abbreviations: ANCA: antineutrophil-cytoplasmic antibodies; CNS: central nervous system, CSF: cerebrospinal fluid; GBS: Guillain-Barré syndrome; EEG: electroencephalography; NCSs: nerve conduction studies; NMDA: N-methyl-D-aspartate; SSA: Sjögren syndrome antigen A; SSB: Sjögren syndrome antigen B; VGKC: voltage-gated potassium channels.

rule out non-infective conditions. Typically, viral encephalitis presents as an acute febrile illness characterised by headache, decreased consciousness, focal seizures and focal neurological signs, as well as CSF markers of inflammation. Within this setting, acute viral encephalitis should be easily separated, on clinical grounds, from disorders causing aseptic meningitis, which manifests with fever, headache, cervical pain and neck stiffness. However, differential diagnosis is not as simple as it may appear. HSE, for example, has generally an abrupt-onset with confusion, severe impairment of episodic and semantic memory, uni- or bilateral temporal seizures, often accompanied by other focal signs. In rare cases, fever may be absent [52]. However, at clinical onset the above clinical features - that reflect involvement of orbito-frontal brain regions and medial temporal lobes - may partly overlap with those observed in transient disorders, such as cerebral concussion, psychiatric conditions, transient global amnesia, and temporal seizures [53].

In addition, a number of static disorders of the temporal lobe, including vascular, neoplastic, and infectious conditions, may closely mimic the florid phenotype of HSE [53]. In particular, autoimmune limbic encephalitis (a subacute neurological syndrome characterised by cognitive impairment, hallucinations, epileptic seizures, depression, and severe memory deficit) must be considered in the differential diagnosis [54]. Further overlap with HSE and, in turn, with limbic encephalitis may occur in patients with steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT), also referred to as Hashimoto's encephalitis (a pleomorphic disorder characterised by high titres of antithyroid antibodies, hyperproteinorrachia, and variable EEG changes) [55]. Patients with SREAT present with acute/subacute myoclonus, altered consciousness, seizures, neuropsychiatric changes, and stroke-like deterioration. Importantly, steroid-responsiveness is typical of SREAT and other encephalopathic disorders belonging to the group of 'nonvasculitic autoimmune meningoencephalitis' (NAIM) [56], such as Sjögren's syndrome and systemic lupus erythematosus.

In limbic encephalitis symptoms are secondary to dysfunction of the limbic system (hippocampus, amygdala, hypothalamus, insular and cingulate cortex) as a consequence of cellular- and/or humoral-mediated autoimmune attack [57]. Limbic encephalitis is usually paraneoplastic, and mostly associated with distant and occult tumours, including small cell lung cancer, testicular seminomas, nonseminomatous germ-cell tumours, and lymphoma. Importantly, in a substantial number of patients, limbic encephalitis is unrelated to cancer and in such cases it may well respond to treatment [58]. The diagnosis of autoimmune limbic encephalitis is unequivocally obtained by demonstrating the presence of circulating autoantibodies recognizing either (a) intracellular onconeuronal antigens (e.g., Hu, Ma2, amphiphysin, and CRMP5/CV2), or (b) neuronal membrane antigens (e.g., N-methyl-D-aspartate receptor, voltage-gated potassium channels) [54, 58].

EEG recordings, magnetic resonance imaging (MRI) of the brain, and CSF examination are the essential first steps for differentiating viral infection from limbic encephalitis, SREAT, NAIM, granulomatous diseases, and primary vasculitis. In cases where MRI is of little help, brain positron emission tomography (PET) scan can provide diagnostic clues. Intriguingly, a limbic encephalitis syndrome, with confusion, altered sleep, temporal seizures, and memory impairment has been reported in immunocompromised, transplanted patients with HHV-6 reactivation [59].

Paraneoplastic conditions, such as brain-stem encephalitis, should be ruled out in the diagnostic workout of patients with rhombencephalitis, a rare brainstem syndrome presenting with myoclonus, ataxia, intention tremor, ocular disturbances, cranial nerve palsies, urinary retention, and, in fatal cases, neurogenic pulmonary oedema and shock. Rhombencephalitis is usually associated with bacterial infections, including *Listeria monocytogenes*, *Mycoplasma pneumoniae*, and *Borrelia burgdorferi*. Sporadic, self-limited, and benign forms have also been attributed to HSV, influenza A, adenovirus, echovirus, and flavivirus infections. In contrast, a rapidly evolving fatal course may occur following EBV and

VZV infections. Recently, fatal outbreaks of enterovirus 71 rhombencephalitis have also been reported [60].

Detection

Viral Sequences in CSF Samples

Although over 100 viral agents can cause encephalitis, in clinical practice identification of the etiologic agent is still rare. Diagnosis was once accomplished by demonstrating intrathecally-produced antiviral antibodies. However, antibody assays are restricted to a few reference laboratories, require considerable time, and interpretation of results is complicated by the low antibody levels found in CSF as well as by passive transfer of antibodies from blood. This makes determination of CSF/serum antibody ratios mandatory.

Nowadays, nucleic acid (NA) technology is capable - in principle - of detecting genomes of any viral species. Molecular methods are characterised by the high sensitivity and rapidity (6 hours or less) that would allow the prompt initiation of antiviral therapy. Indeed in CSF samples NA technology retains sensitivity for some time after initiation of antivirals [61]. Since quantification of genome copy numbers may be a marker of disease severity and often predicts clinical outcome, quantitative methods are being increasingly applied to estimate the viral load and to monitor, for some viruses (e.g., HSV), the effects of antivirals [62].

Laboratory diagnosis begins with the NA extraction from CSF (rarely from brain biopsy). To this end, commercial methods are preferred for their effectiveness, and standardisation. Recently introduced, automated methods based on NA-binding magnetic particles are characterised by high purity of extracted NA. In our experience, manual methods continue to be satisfactory due to the low numbers of specimens usually submitted to laboratories for suspected encephalitis. NAs are usually extracted with methods dedicated to either DNA or RNA.

While most laboratories use NA extraction kits with similar performance, gene amplification methods are highly diverse. The polymerase chain reaction (PCR) is the most common technique, but - for any given agent - several primer sets targeting different genes have been proposed by different authors. In addition, protocols differ with regard to reaction conditions, techniques for confirming amplified products, virus strains used as positive controls. Over the last few years, commercial methods for the most common viral agents have been introduced by major diagnostic Companies and are being evaluated the world over. Table **3** lists encephalitis agents (some not examined in this review) with selected diagnostic molecular methods and relevant references. A positive PCR result must always be considered in conjunction with clinical symptoms and signs prior to establishing a diagnosis. A negative RT-PCR result indicates only the absence of the agent being investigated in the sample, and does not exclude the suspected diagnosis. Equivocal results are those that fall between the lowest limit of detection and the background level. These can not be determined to be positive or negative. Qualitative PCR and RT-PCR are the amplification methods most frequently used. Both use thermostable DNA polymerases, specific primer sets, and dedicated thermocycling profiles. Amplicons are detected by gel electrophoresis and/or by hybridisation with a labelled probe. Real-time amplification (a technique that allows quantification of amplified products) needs one or more primer sets plus photo-emitting probe(s) or, alternatively, a primer set plus a fluorescent dye that binds double-stranded DNA (e.g., SYBR Green). A recently proposed method is the loop-mediated isothermal amplification (LAMP) assay. LAMP uses six or more primers that target a single gene. Its efficacy, sensitivity, and simplicity have been proved for CMV [63].

In clinical practice, laboratory diagnosis of viral encephalitis remains difficult due to the need of detecting a wide variety of agents in a short time. Multiplex PCR methods (i.e., methods capable of detecting multiple targets in a single test) have been proposed [64-67], but are rarely used. Notably, one system would allow the simultaneous detection of many enterovirus types, some herpesviruses and selected flaviviruses [65]. The specificity and sensitivity of this system, however, have not been documented.

Neuroimaging

Neuroimaging plays an important role in detecting inflammatory lesions of brain and meninges, since visualisation of typical lesion patterns does contribute to diagnosis.

In HSE brain CT scan reveals hypodense, contrast-enhancing lesions in antero- and medial temporal and in inferior frontal regions. Oedema and mass effect occur in 80% of cases. Brain MRI is superior to CT scan in detecting necrosis in the orbitofrontal and medial temporal lobe, and in the insulae. Necrosis is demonstrated by increased signalling within the first 48 hours on T2-weighted (T2WI) or fluid-attenuated inversion recovery (FLAIR) sequences [68] (Fig. **1**), with decreased T1 signal and variable enhancement. Diffusion-weighted imaging (DWI) is even more sensitive than T2WI or FLAIR in detecting early cortical lesions in HSE, both in infants and adults [69]. Diffusion abnormalities disappear within 14 days after onset, whereas hyperintensities on T2WI persist [69].

In HHV-6 encephalitis, MRI shows predominantly mesial temporal lobe abnormalities, including T2 signal hyperintensities and early volume loss in the hippocampus [70, 71]. DW-MRI is a promising tool for early detection and for outcome prediction [72].

EBV encephalitis in adult immunocompetent patients produces normal or mildly swollen brain on MRI, while in infants, due to characteristic tropism to deep grey nuclei, a pattern of increased signal on T2WI in the bilateral thalami and basal ganglia is observed.

In acute measles encephalitis, T2WI reveals cortical oedema and bilateral symmetric hyperintense lesions in putamen and caudate nuclei as well as in centrum semiovale. Contrast enhancement may appear in cortical areas and leptomeninges [73]. In early stages of SSPE, MR spectroscopy demonstrates increased choline/creatine ratios suggestive of asymmetrical inflammation in parieto-occipital corticosubcortical regions. In the late stages, conventional MRI discloses symmetrical periventricular hyperintense changes on T2WI [74].

In patients with TBE, pathological T2-weighted and FLAIR MRI changes can be observed in almost 20% of

Table 3. Molecular Methods for Detecting the Main Agents of Viral Encephalitis [References in Square Brackets]

(a) DNA Viruses

	Virus	Gene Amplification Method				
		Qualitative PCR	Quantitative PCR	Multiplex PCR	Other Techniques	
Herpesviridae	**Herpes Simplex Virus (HSV)**	Target gene: *DNA polymerase* [61]	Target gene: *US4* [99]	Target region: herpesvirus *DNA polymerase*. Simultaneous detection of enteroviruses, herpesviruses and flaviviruses. Broadly reactive primers for the three family, then DNA probe array to differentiate virus species [65]	Target gene: *DNA polymerase.* PCR and chemiluminescent dot blot hybridization or hetroduplex mobility shift assay. Detection of herpesviruses and species identification [101]	DNA microarray: 38 target genes for 13 encephalitis-producing viruses [104]
	Varicella-Zoster Virus (VZV)	Target gene: *gp29* [61]	Target region: *ORF38* [99]			
	Epstein-Barr Virus (EBV)	Target gene: *gp220* [61]	Target region: *BNT143* [99]			
	Cytomegalo Virus (CMV)	Target gene: *MIE* [61]	Target genes: CMV, *UL54*; HHV-6, *U56* [100]		Target gene: *gB*. LAMP [63]	
	Human Herpes Virus-6 (HHV-6)	Target gene: *U86, U95* [98]			Target gene: *U67*. Viral load in CSF, whole blood and serum. Differentiation between primary infection and viral integration. Distinction of 6A/ 6B variants through restriction analysis [102]	
Polyomaviridae	**JCV**		Target gene: *late mRNA* [99]	Target gene: *T large antigen* [64]	PCR and colorimetric hybridization assay for differential detection of JC and BK virus genomes [103]. Commercially available.	
	BKV		Target gene: *VP3* [99]			
Adenoviridae	**Adenovirus**		Target region: L2p5 precursor, E1B, L5 pVI, hexon gene [99]			

(b) RNA Viruses

	Virus	Gene Amplification Method			
		Qualitative PCR	Quantitative PCR	Multiplex PCR	Other Techniques
Picornaviridae	**Enteroviruses (EV) Polio and Several Non-Polio EV are Included EV71**	Target gene: *VP3-VP1*. Seminested PCR. Species identification by sequencing [105] Target genes: *5'UTR* for detection [106]. Species identification by microarray technology [107]	Target region: 5'UTR. Detection of all EV [109]	Target region: 5'UTR. Simultaneous detection of enteroviruses, herpesviruses and flaviviruses. Broadly reactive primers for the three family, then DNA probe array to differentiate virus species [65]	
Retroviridae	**Human Immunodeficiency Virus-1/2 (HIV-1/2)**	Commercial methods	Commercial methods [110]		Real-time PCR-based point mutation assay for detection of drug resistance mutations [115]
Rabdhoviridae	**Rabies**		Target gene: *N*. Quantitative detection and distinction of different genotypes [111]		
Paramyxoviridae	**Mumps**	Target gene: *SH*. Nested PCR and sequencing to perform genetic classification [108]	Target gene: *SH*. One step assay with internal controls for nucleic acid extraction and PCR inhibitors [112]	Target gene: *HN;* RT-LAMP [113]	DNA microarray: 38 target genes for 13 encephalitis-producing viruses [104]
	Measles			Target gene: *N*. Real Time ARMS PCR: six primer sets; genotypes differentiated by melting curve analysis [114]	

(Table 3) contd…..

(c) Arbovirus Group (RNA Agents)

	Virus		Gene Amplification Method				
			Qualitative PCR	Quantitative PCR	Multiplex PCR		Other Techniques
Togaviridae	**Eastern Equine Encephalitis (EEE)**		Target gene: *eeevgp2* coding for P123, nsP3, nsP4 [116]	Target gene: *eeevgp3* coding for PE2, E2 [118]	Target gene: *nsP1,* togaviruses; *NS5*, flaviviruses [122]		Target gene: *eeevgp3* coding for PE2, E2. NASBA [116]
	Western equine encephalitis (WEE)		Target gene: *weevgp1* coding for P123, nsP3 [116]	Target gene: *weevgp2* coding for E1 [118]			Target gene: *weevgp* coding for *2*E2. NASBA [116]
	Venezuelan equine encephalitis (VEE)			Target gene: *E1, E2, 6K* [120]			
Flaviviridae	**St Louis Encephalitis (SLE)**		Target region: capsid/preM [61]	Target genes: *slevgp1, SLE; wnvgp1*, WNV [66]		Target region: NS5. Simultaneous detection of enteroviruses, herpesviruses and selected flaviviruses. Broadly reactive primers for the three families, then DNA array to differentiate virus species [65]	
	West Nile Virus (WNV)		Target gene: *NS3* [61]		Target genes: *wnvgp1*, WNV; *pol*, JE. Real time PCR; probe common to JE and WNV [123]		
	Japanese Encephalitis (JE)		Target gene: *E.* One step RT-PCR [117]				Target gene: *E.* RT-LAMP [117]
	Powassan Virus (POW)		Target gene: *NS* [61]				
	Murray Valley Encephalitis (MVE)						
	Tick-Borne Encephalitis Viruses (TBEV)	**Far Eastern Subtype (FE-TBEV)**	Target region: *NS5.* RT-nested PCR [118]	Target region: 3' UTR [121]	Target gene: *E.* Three primer sets [67]		
		Siberian Subtype (S-TBEV)					
		Western Subtype (W-TBEV)					
Bunyaviridae	**California Group**	**California Virus**	Target region: *middle RNA* [61]				Target gene: *M.* NASBA [116]
		La Crosse Virus (LAC)					
	Sandfly Fever Group	**Toscana Virus (TOSV)**	Target region: S and L segment [119]				

Abbreviations: PCR: polymerase chain reaction; RT-PCR: reverse transcription PCR; LAMP: loop-mediated isothermal amplification; NASBA: nucleic acid sequence-based amplification; Real time ARMS PCR: real-time amplification refractory mutation system PCR.

cases. Bilateral involvement of thalamus, and abnormalities of cerebellum, brain stem, basal ganglia, spinal cord are observed [75, 76].

Although the diagnosis of rabies is mostly clinical, MRI can be helpful. Both paralytic and encephalitic forms have similar distribution of mild T2 hyperintensities involving the brain stem, hippocampus, hypothalamus, deep and subcortical white matter, cerebral cortical grey matter, and basal ganglia. Alterations vary in different stages of the disease [77].

Management

The general management is essentially supportive and in severe cases must be in a high dependency or intensive care unit. Focal and generalised seizures need to be treated effectively with anticonvulsants intravenously. Raised intracranial pressure must be treated with intravenous mannitol and/or steroids. In the case of rapidly increasing intracranial pressure with clinical deterioration unresponsive to medical treatment, surgical decompression can be lifesaving. Other complications such as secondary bacterial infections, aspiration pneumonia, respiratory failure, cardiac abnormalities, fluid and electrolyte imbalance must be detected early and treated appropriately.

Herpesvirus-related encephalitis can be treated with a potentially effective antiviral therapy. The administration of acyclovir (within 48 hours of the onset of symptoms) at a dose of 10 mg/kg bw intravenously thrice per day for 14 days (21 days in immunocompromised hosts) reduces both the mortality rate and long-term severe neurological consequences in patients with HSE [78, 79]. However, mortality remains high (14%, increasing to 25.4% by the end of the

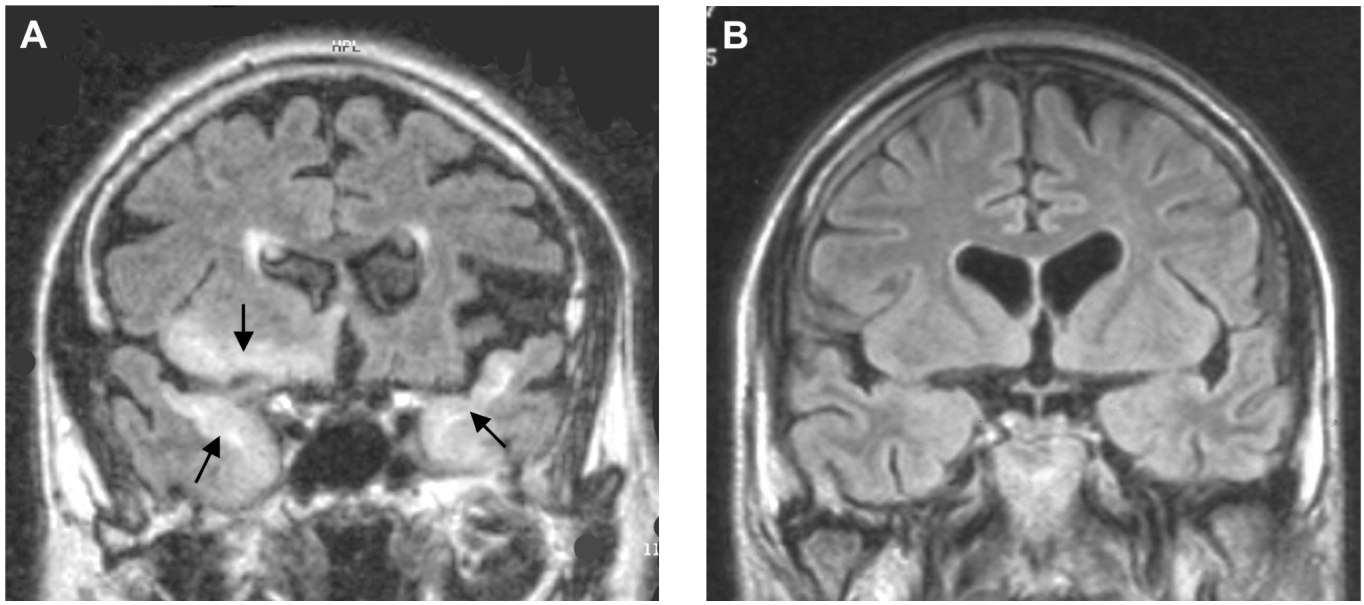

Fig. (1). Coronal fluid-attenuated inversion recovery (FLAIR) MRI scan demonstrates: bilateral involvement of medial temporal lobes and right orbitofrontal region (*arrows*) in one case of HSV-1 encephalitis (**A**), and normal image of one age-matched control (**B**).

first year after treatment in Sweden over an 11 years period) [80], especially in patients with Glasgow coma score lower than 6. Patients under 30 years of age and with a Glasgow coma score above 10 have the best outcome. In immunocompetent patients, herpesvirus resistance is rare and generally does not correlate with clinical outcome [81]. High dose steroids may be helpful in selected cases in children, especially when a biphasic course or relapses occur [82, 83].

In VZV encephalitis, the treatment benefit of acyclovir used routinely in immunologically competent children has not been proved. IV methylprednisolone can be given to patients who present with neurological deficits with a delay after onset (> 7days) as these are likely to be cases of ADEM.

EBV encephalitis has no effective therapy (antiviral agents, immunoglobulins and steroids are ineffective). Different antivirals (ganciclovir, foscarnet and cidofovir) have been reportedly useful in few cases of HHV-6 encephalitis, but no clinical trials have been published [84]. No therapy exists for enteroviral encephalitis, although the broad-spectrum antipicornavirus drug pleconaril might be an option in the future [85].

Various antiviral and immunomodulatory agents have been used occasionally to treat SSPE with contradictory results. Encouraging results were reported in a multicenter study with a 6-month follow-up evaluating oral inosiplex (isoprinosine) alone or combined with intraventricular IFN-α2b therapy [86], but other studies are needed to confirm these data.

Rabies encephalitis is progressive and fatal, and therapy is largely palliative. Although survival has been reported in isolated cases of symptomatic patients, all of those patients had received either pre-or postexposure prophylaxis and none had positive test results for rabies virus [87-92]. Disabling neurologic sequelae followed in nearly all of these survivors. In 2005, Willoughby and colleagues described the survival of a nonvaccinated young girl with encephalitic rabies following an aggressive treatment that included therapeutic coma, antiviral therapy and intensive care support [93]. The strategy, known as the Milwaukee Protocol, involves administration of ketamine, midazolam, amantadine, ribavirin and phenobarbitol, and is based on the idea that, given sufficient time, antiviral and antiexcitatory therapy will permit for viral clearance and clinical recovery. However, in the case described by Willoughby and colleagues, the rabies virus was never isolated and diagnosis was based on patient history, clinical findings and detection of anti-rabies virus antibodies in the blood and cerebrospinal fluid. Six additional cases of human rabies treated with the Milwaukee Protocol have been described [94-97]. None of the patients had received postexposure prophylaxis, all patients presented with clinical disease, and none survived. In spite of the detection of anti-rabies virus antibodies in these patients and of evidence of viral clearance, autopsies of most of the patients still revealed the presence of the rabies virus. In conclusion, at present rabies encephalitis must continue to be regarded as an incurable condition.

CONCLUSIONS

Viral encephalitis continues to pose many difficulties with regard to diagnosis and management. In spite of the sophisticated imaging and molecular methods today available, too often a cause is not identified. Supportive therapy to control seizures, respiratory arrest, cerebral oedema and to prevent deep vein thrombosis, aspiration pneumonia, bacterial infections, gastrointestinal bleeding as well as disseminated intravascular coagulation still represent the mainstay of treatment. The pathogenesis remains largely elusive, and only one form (HSE) can benefit from treatment. The use of steroids as adjunctive therapy continues to be controversial, although a number of reports have shown clinical benefits. Novel methods have been recently proposed for viral identification; thus it is hoped that random DNA amplification coupled to other molecular techniques [87,88] will allow detection of the multiple agents of encephalitis.

Much needs to be done to improve the prognosis of viral encephalitis. Unfortunately, the rarity of the disease will likely prevent substantial efforts by drug companies to develop novel and more targeted therapies.

SEARCH STRATEGY AND SELECTION CRITERIA

Data for this review were identified by searches of Medline, Current Contents, and references from relevant articles; numerous articles were identified through searches of the extensive files of the authors. Search terms were "encephalitis" or "viral encephalitis" and “etiology”, “pathogenesis”, “diagnosis”, “symptoms”, “investigation”, “radiology”, “computerised tomography”, “nuclear magnetic resonance”, “positron emission tomography”, “single photon emission tomography”, “pathology”, “therapy”, “antiviral therapy”, “polymerase chain reaction”, “virology”, “cerebrospinal fluid”, “EBV”, “enteroviruses”, “herpes simplex virus”, “herpes zoster virus”, “HHV-6”, “measles”, “rabies”, “SSPE”, “West Nile Virus” or “tick borne”. Only English language papers published from 1960 to September 2008 were reviewed.

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