

INTRODUCTION

Encephalitis is an inflammatory process that affects the parenchyma of the brain, usually present as a diffuse and, or focal neuropsychological dysfunction, indicates that the predominant clinical syndrome arises from infection and inflammatory reaction in the parenchyma of the brain rather than in the leptomeninges. When both the leptomeninges and brain parenchyma are involved, the term meningoencephalitis is used. ⁽¹⁾

Viral encephalitis is a medical emergency. The spectrum of brain involvement and the prognosis are dependent mainly on the specific pathogen and the immunological state of the host. Although specific therapy is limited to only several viral agents, correct immediate diagnosis and introduction of symptomatic and specific therapy has a dramatic influence upon survival and reduces the extent of permanent brain injury in survivors. ⁽²⁾

The virus infects the brain causing immune mediated inflammatory changes and destruction of gray matter that is usually accompanied by inflammation of the adjacent meninges. ⁽³⁻⁶⁾

Epidemiology:

Encephalitis can be sporadic or epidemic and differences in epidemiological and etiological characteristics further help define the various categories of encephalitis. ⁽³⁾

The global reported incidence of encephalitis varies according to the location, population studied, and differences in case definitions and research methods; however, the reported incidence in western settings ranges from 0.7 to 13.8 per 100,000 for all ages, being approximately 0.7-12.6 per 100,000 for adults and 10.5-13.8 per 100,000 for children. ^(7, 8)

Incidence of encephalitis in Egypt was 1394 per 70,000,000 population. ⁽⁹⁾ Herpes Simplex Virus (HSV) encephalitis is the most commonly diagnosed viral encephalitis in industrialized nations, with an annual incidence of 1 in 250,000 to 500,000. ^(10, 11)

Among less common causes of viral encephalitis, varicella-zoster encephalitis which caused by cytomegalovirus (CMV) has an incidence of 1 in 200 (infected persons). Measles produces two devastating forms of encephalitis: postinfectious, which occurs in about 1 in 1000 infected persons, and Subacute sclerosing panencephalitis (SSPE), occurring in about 1 in 100,000 infected patients. ^(1, 12)

In chickenpox, the rate of encephalitis is approximately 0.3/1000 cases, ⁽¹³⁾ and the case-fatality rate is approximately 17 %. ⁽¹⁴⁾ of patients with herpes zoster, 0.5 to 5 % has encephalitis. ⁽¹⁵⁾

Enteroviruses most often cause aseptic meningitis but can also be an important cause of encephalitis. ⁽¹⁶⁾ Among the other causes, encephalitis associated with antibodies to the voltage-gated potassium channel complex, or N-Methyl-D-Aspartate Antibody (NMDA) receptors are increasingly recognised. ⁽¹⁷⁾

The enteroviruses are responsible for up to 20% of identifiable causes of viral encephalitis. In the single largest report of enterovirus-related encephalitis, 73% of

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confirmed cases occurred in individuals younger than 20 years, including about 40% of cases in patients younger than 10 years. Among enteroviral encephalitides, enterovirus 71 is uniquely associated with severe brain stem encephalitis (rhombencephalitis), primarily in children. ⁽¹⁸⁾

Mumps meningoencephalitis affects male more often than female. ⁽¹⁹⁾ In infectious mononucleosis, encephalitis occurs in less than 1 % of cases. Most patients with Epstein-Barr virus encephalitis are adolescents and young adults, and although patients typically present 1 to 3 weeks after the onset of mononucleosis syndrome, encephalitis may be the presenting complaint in Epstein-Barr virus infection. ⁽²⁰⁾

The age specific incidence is bimodal, with peaks in the young and the elderly. Most HSV encephalitis is due to HSV-1, but about 10% is caused by HSV-2. The latter typically occurs in immunocompromised individuals and neonates, in whom it can cause a disseminated infection. ⁽¹⁶⁾

Etiology

The specific etiology as many as 32% to 75% of acute encephalitis cases remains unknown even after a thorough etiological workup. ^(21, 22)

Table (1): Etiologic agents in acute encephalitis, meningo-encephalitis, and acute illnesses with an encephalitic component. ⁽¹⁶⁾

Category	Causes
(1) Viruses • Spred person to person only	1- Herpes simplex virus types 1 and 2 2- Varicella zoster 3- Eptein – Barr 4- Cytomegalovirus 5- Human herpes virus type 6 6- Human herpes virus type 7 7- Enteroviruses 8- Reoviruses 9- Influenza A and B 10- Respiratory syncytial 11- Parainfluenza 1-3 12- Adenovirus 13- Rubella 14- Human coronavirus 15- Mumps 16- Measles 17- Variola 18- Hepatitis A,B,C 19- Human parvovirus 20- Rotavirus 21- BK virus , John Cunningham virus (JCV) family of polyomavirus

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<ul style="list-style-type: none"> • Spread to humans by mosquitoes or ticks 	<ul style="list-style-type: none"> • Arboviruses :- 1- Saint Louis virus 2- West Nile virus 3- Eastern equine virus 4- Western equine virus 5- Venezuelan equine virus 6- California virus 7- Powassan virus 8- Colorado tick fever
<ul style="list-style-type: none"> • Spread by warm blooded mammals 	<ol style="list-style-type: none"> 1- Rabies 2- Simian herpes virus (herpesvirus B) 3- Lymphocytic choriomeningitis 4- Encephalomyocarditis 5- Vesicular stomatitis 6- Equine morbillivirus (hendra virus) 7- Nipah 8- Monkeypox
(2) Bacteria	<ol style="list-style-type: none"> 1- Haemophilus influenza <ul style="list-style-type: none"> • Neisseria meningitides • Streptococcus pneumonia • Mycobacterium tuberculosis and other bacterial meningitides often have an encephalitic component 2- Spirochetal infections :- <ul style="list-style-type: none"> • Treponema pallidum • Leptospira • Borrelia burgdorferi and other Borrelia spp. Infections 3- Brucella spp. 4- Actinomyces and Nocardia 5- Bartonella henselae 6- Listeria monocytogenes
(3) Fungal	<ol style="list-style-type: none"> 1- Coccidioides immitis 2- Cryptococcus neoformans and other fungal meningitides often have a encephalitic componenet
(4) Protozoal	<ol style="list-style-type: none"> 1- Plasmodium spp. 2- Trypanosoma spp. 3- Naegleria spp. 4- Acanthamoeba spp. 5- Balamutbia mandrillaris 6- Toxoplasma gondii
(5) Helminthes	<ol style="list-style-type: none"> 1- Trichinella spiralis 2- Schistosoma spp. 3- Strongyloides stercoralis 4- Baylisascaris procyonis

(6) Others	1- Chlamydia psittaci 2- Chlamydia pneumonia 3- Rickettsial infections :- <ul style="list-style-type: none">• Rocky Mountain spotted fever• Ehrlichiosis• Q fever• Typhus 4- Mycoplasma infections :- <ul style="list-style-type: none">• Mycoplasma pneumonia• Mycoplasma hominis
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Pathogenesis

In general, the virus replicates outside the central nervous system (CNS) and gains entry either by hematogenous spread or by traveling along neural (rabies, HSV, varicella zoster virus VZV) and olfactory (HSV) pathways.⁽²³⁾ Once cross the blood brain barrier, the virus enters neural cells, with resultant disruption in cell functioning, perivascular congestion, hemorrhage, and inflammatory response diffusely affecting gray matter disproportionately to white matter. Focal pathology is the result of neuron cell membrane receptors found only in specific portions of the brain and accounts for regional tropism found with some viruses. For example, HSV has predilection for the inferior and medial temporal lobes.⁽²⁴⁾

Transmission of virus into the CNS can be haematogenous, resulting from changes in the vasculature that enable virus to cross the blood-brain-barrier either directly or within infected cells crossing the endothelium. Alternatively entry can be neuronal of which rabies virus and herpes viruses are the best characterized.⁽²⁵⁾

Entry by the haematogenous route there is likely to have been prior virus replication in the periphery often resulting in a detectable viraemia and an opportunity for systemic immune activation and an adaptive response demonstrable by the presence of anti-virus antibodies in serum. There is usually a febrile phase that often resolves and the appearance of virus-neutralising antibodies in the blood that rapidly controls viraemia.⁽²⁶⁾

Entry by the neuronal route may occur with minimal virus replication in the periphery and therefore the first contact between the immune response and infecting virus occurs within the CNS and can provide a direct challenge to the survival of the host. In this case, the earliest response to the appearance of virus within the CNS is the innate-immune response.^(27, 28)

The innate immune responses are activated by recognition of pathogen-associated molecular patterns (PAMPs).⁽²⁹⁾ For viruses, this includes surface glycoproteins and structures associated with single- or double-stranded ribonucleic acid (RNA). Recognition is mediated by a number of protein families, the most prominent being the toll-like receptors (TLRs), NOD-like receptors and retinoic acid-inducible gene 1(RIG-I) helicases.^(30, 31)

These receptors are expressed in many compartments of the cell and trigger the production of type I interferons, a key element in controlling virus replication and spread.⁽³²⁾ Type 2 interferons are produced exclusively by lymphoid cells, which are

absent early in infection but contribute to later control of pathogens. Interferons in turn activate the up-regulation of a large number of proteins that act to control infection at the cellular level and attract immune effector cells. ^(33, 34)

There are three mechanisms of direct inhibition of virus replication have been identified. Activation of protein kinase R (PKR) in response to double stranded RNA (e.g. virus replication intermediates) which inhibits eukaryotic translational factor 2 that in turn restricts synthesis of viral proteins. Activation of 2'5' oligoadenylate synthetase (OAS), which activates RNase L and in turn degrades viral RNA. ⁽³⁵⁾

Finally, the Mx family of proteins are activated that target nucleocapsids (viral structures that contain the genome bound to viral proteins including the nucleoprotein, a protein encoded by many viruses). ^(36, 37)

Whilst the CNS has unique immunological status there is increasing evidence that there is a vigorous innate immune response to viral infection of cells within it. ⁽³⁸⁾

Such as, Toll like receptors (TLRs) are selectively up-regulated in the brain in response to infection with different viruses. ⁽³⁹⁾ Neurons can produce a range of innate immune-associated proteins including type 1 interferons in response to infection with rabies virus ⁽⁴⁰⁾ and La Crosse virus. ⁽⁴¹⁾

Microglial cells are capable of producing a wide range of inflammatory mediators in response to activation ⁽⁴²⁾ or direct infection. ⁽⁴³⁾ Viruses have evolved a range of mechanisms to subvert the innate immune response, particularly through inhibition of the interferon system. ⁽³²⁾ These often involve direct interaction of virally expressed proteins with signaling intermediates that would normally trigger the expression of interferon or interferon-induced proteins.

Whilst innate immune inhibition occurs within the cell, neuro invasion by viruses rapidly induces immune responses across the CNS. A key family of proteins produced in response to infection is the chemokines whose primary role is to attract immune effector cells to sites of infection. Many cytokines are toxic to neurons and oligodendrocytes including interleukin 1 β (IL1 β), tumor necrosis factor - α (TNF- α) and interferon- γ . ⁽⁴⁴⁾

Uncontrolled cytokine expression will lead to tissue damage, a situation that occurs in auto-immune mediated encephalopathies such as multiple sclerosis and Alzheimer's disease. Innate immune responses are suppressed by transforming growth factor - β (TGF- β), produced by resting and active astrocytes. ⁽⁴⁵⁾ During inflammation, the family of suppressors of cytokine signaling (SOCS) proteins is induced by cytokine expression and block signal transduction activated by these same cytokines providing feedback inhibition of inflammation. ⁽⁴⁶⁾ There is also evidence that these proteins are selectively expressed in the brain to control inflammation in response to viral infection. ⁽⁴⁷⁾

Encephalitis is classified as:-

(1) Primary encephalitis :

In which encephalitis is the major manifestation. Symptoms are caused by direct invasion and replication of an infectious agent in the central nervous system (CNS), resulting in objective clinical evidence of cerebral or cerebellar dysfunction.⁽⁴⁸⁾

(2) Post infectious or Para- infectious encephalitis :

It occurs after or in combination with other illnesses that are not CNS illnesses, or after a vaccine has been administered.

Manifestations may be mediated immunologically. When neurologic clinical findings suggest encephalitis but inflammation of the brain has not occurred (e.g., in Reye syndrome), the condition is identified by the less specific term encephalopathy. Frequently, when encephalitis or meningoencephalitis occurs, other areas of the nervous system, such as the spinal cord (myelitis), nerve roots (radiculitis), and nerves (neuritis), also are involved.⁽⁴⁹⁾

Infectious encephalitis and post-infectious or post-immunization encephalitis or encephalomyelitis: (e.g., acute disseminated encephalomyelitis [ADEM]), which may be mediated by an immunologic response to an antecedent antigenic stimulus from an infecting microorganism or immunization.

Non-infectious CNS diseases: (e.g., vasculitis, collagen vascular disorders, and paraneoplastic syndromes) can have clinical presentations similar to those of infectious causes of encephalitis and should also be considered in the differential diagnosis.⁽⁵⁰⁾

Clinical picture

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 lymphadenopathy, nausea or vomiting.⁽⁵¹⁾

After a few days, symptoms of CNS involvement become manifest with altered mental status, considerable irritability and agitation, personality changes; seizures (focal or generalized) 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 years. Young children may have a stormy course for several days because of severe cerebral oedema.⁽⁵¹⁾

History

A detailed history needed to be taken from the relatives since a patient with encephalitis is likely to be confused, disoriented, delirious, or comatose. Both the geographical distribution and seasonal occurrence may offer important clues.⁽⁵²⁾

It is essential that a history should always be sought for recent ticks bites (e.g Ixodes species in tickborne encephalitis virus) and possible contact with individuals suffering from infectious diseases. The underlying medical condition is also relevant since immunosuppressed individuals are more susceptible to certain specific infective encephalitis, for example, listeriosis, cryptococcus, and cytomegalovirus. Cytomegalovirus encephalitis is common in human immunodeficiency virus (HIV) infected patients, particularly in neonates.⁽⁵³⁾

The mode of onset and progression of the viral illness may provide valuable clues to the aetiology, for example, biphasic course of the enterovirus infection.⁽⁵⁴⁾

Clinical signs

(A) General examination

Start by examination of the skin as some etiological agents of encephalitis also cause dermatological lesions. A prime example is HSV which is the most common viral cause of encephalitis causes herpetic skin lesions,⁽⁵⁵⁾ also in Epstein barr virus EBV⁽⁵⁶⁾ in which jaundice and oral petechiae can be observed.⁽⁵⁶⁾ Ocular symptoms should be observed as in HSV infection the patient can develop keratoconjunctivitis.⁽⁵⁷⁾ Also in EBV infection a periorbital oedema may be noted.⁽⁵⁸⁾ Examination of the oral cavity as in HSV ulcers on the buccal mucosa and the tongue are observed.⁽⁵⁹⁾ In West Nile Virus (WNV) patients suffer from lymphadenopathy so the tonsils should be examined for any indications of tonsillitis.⁽⁶⁰⁾ Periauricular lymph nodes are enlarged during WNV infection.⁽⁶¹⁾

(B) Neurological examination

Neurological signs in acute encephalitis do not reliably identify the underlying aetiology despite the propensity of certain neurotropic viruses to affect specific focal areas of the central nervous system.

The most commonly reported focal abnormalities are hemiparesis, aphasia, ataxia, pyramidal signs (brisk tendon reflexes and extensor plantar responses), cranial nerve deficits (oculomotor and facial), involuntary movements (myoclonus and tremors), and partial seizures.⁽⁶²⁾

The evolution of the clinical signs will depend on the virus, the age, and the immune status of the patient. In general, the very young and the very old have the most serious clinical manifestations of encephalitis. A constellation of fronto-temporal signs with aphasia, personality change, and focal seizures is characteristic of HSE. The presence of multifocal lower motor neuron signs in a febrile patient might indicate poliomyelitis.⁽⁶²⁾

Symptoms of autonomic or hypothalamic dysfunction may also be seen in acute encephalitis. These include loss of temperature and vasomotor control (dysautonomia), diabetes insipidus, and the syndrome of inappropriate secretion of antidiuretic hormone.⁽⁶²⁾

Diagnosis

I- Laboratory Investigations

(1) General investigation

- Peripheral blood count and cellular morphology are helpful in separating viral from non-viral infections. Lymphocytosis in the peripheral blood is common in viral encephalitis.
- Erythrocyte sedimentation rate (ESR) is another non-specific test that is usually within normal range in viral infections.
- Others such as, blood cultures, belong to the general work-up of febrile disease.⁽²⁾
- Serum Sodium → Hyponatraemia due to the syndrome of inappropriate antidiuretic hormone (SIADH) is common in encephalitis.⁽⁶³⁾
- Serum amylase → a raised serum amylase is common in mumps virus infection.⁽⁶³⁾

If there has been a respiratory component to the presentation check for cold agglutinins, which occur in mycoplasma infection, and order appropriate serological investigations for mycoplasma and Chlamydia (atypical respiratory infections).

- The human immunodeficiency virus (HIV) testing should also be considered, especially if the cause of CNS infection is uncertain.⁽⁶³⁾

(2) Lumbar puncture

A lumbar puncture is an essential investigation because the initial cerebrospinal fluid (CSF) findings (especially the cell count, differential and glucose ratio) reveal firstly whether or not there is infection, and secondly whether it is likely to be bacterial or viral infection, which thus informs the initial antimicrobial therapy.⁽⁶³⁾ (See table 2 shows: Typical cerebrospinal fluid finding in CNS infections).⁽⁶⁴⁾

Table (2): Typical cerebrospinal fluid findings in central nervous system infections. ⁽⁶⁴⁾

	Viral meningo-encephalitis	Acute bacterial meningitis	Tuberculous meningitis	Fungal	Normal
Opening pressure	Normal/high	High	High	High-very high	10-20 cm*
Colour	"Gin" clear	Cloudy	Cloudy/yellow	Clear/cloudy	Clear
Cells/mm ³	Slightly increased	High-very high	Slightly increased	Normal-high	<5†
Differential	Lymphocytes	Neutrophils	Lymphocytes	Lymphocytes	Lymphocytes
CSF/plasma glucose ratio	Normal	Low	Low-very low (<30%)	Normal-low	66%‡
Protein (g/l)	Normal-high 0.5-1	High >1	High-very high 1.0-5.0	Normal-high 0.2-5.0	<0.45†

*Normal CSF opening pressure is generally <20 cm for adults, <10 cm for children below age 8, but may be as high as 25 cm

†A bloody tap will falsely elevate the CSF white cell count and protein. To correct for a bloody tap, subtract 1 white cell for every 700 red blood cells/mm³ in the CSF, and 0.1 g/dl of protein for every 1000 red blood cells.

‡A normal glucose ratio is usually quoted as 66%, though only values below 50% are likely to be significant.

Some important exceptions:

- In viral CNS infections, an early lumbar puncture may give predominantly neutrophils, or there may be no cells in early or late lumbar punctures.
- In patients with acute bacterial meningitis that has been partially pre-treated with antibiotics (or patients <1 year old) the CSF cell count may not be very high and may be mostly lymphocytic.
- Tuberculous meningitis may have predominant CSF polymorphs early on.
- *Listeria* can give a similar CSF picture to tuberculous meningitis, but the history is shorter.
- CSF findings in bacterial abscesses range from near normal to purulent, depending on location of the abscess, and whether there is associated meningitis, or rupture.
- A cryptococcal antigen test and India ink stain should be performed on the CSF of all patients in whom cryptococcus is possible.

Although a lymphocytic CSF pleocytosis is typical of viral CNS infections, bacterial infection can give a similar picture particularly in tuberculosis, listeriosis, brucellosis and partially treated acute bacterial meningitis. Usually the clinical setting and other CSF parameters (low glucose ratio and higher protein) will suggest these possibilities. ^(65, 66)

CSF lactate may be helpful in distinguishing bacterial meningitis from viral CNS infections; in particular, a CSF lactate of <2 mmol/l is said to rule out bacterial disease. ^(65, 66)

Following a traumatic tap white blood cells and protein from the blood can contaminate the CSF. ⁽⁶⁷⁾

The white cell count and protein can be approximately corrected for the number of red cells in the CSF by subtracting 1 white cell for every 500-1000 red blood cells in the CSF, and 0.1 g/dl protein for every 100 red blood cells. This approximation will suffice in most circumstances, though more complicated formulae allowing for anaemia etc are available. ⁽⁶⁷⁾

Contraindications to immediate lumbar puncture (See Table 3: Contraindications to an immediate lumbar puncture in patients with suspected CNS infections). ⁽⁶⁸⁻⁷⁰⁾

Table (3): Contraindications to an immediate lumbar puncture in patients with suspected CNS infections. ⁽⁶⁸⁻⁷⁰⁾

Imaging needed before lumbar puncture (to exclude brain shift, swelling, or space occupying lesion)

- Moderate to severe impairment of consciousness (GCS < 13)^a or fall in GCS of >2
- Focal neurological signs (including unequal, dilated or poorly responsive pupils)
- Abnormal posture or posturing
- Papilloedema
- After seizures until stabilised
- Relative bradycardia with hypertension
- Abnormal 'doll's eye' movements
- Immunocompromise

Other contraindications

- Systemic shock
- Coagulation abnormalities:
 - Coagulation results (if obtained) outside the normal range
 - Platelet count <100 × 10⁹/L
 - Anticoagulant therapy
- Local infection at the lumbar puncture site
- Respiratory insufficiency
- Suspected meningococcal septicaemia (extensive or spreading purpura)

^aThere is no agreement on the depth of coma that necessitates imaging before lumbar puncture; some argue Glasgow coma score < 12, others Glasgow coma score < 9.

- Patients on warfarin should be treated with heparin instead, and this stopped before lumbar puncture.
- Consider imaging before lumbar puncture in patients with known severe immunocompromise (e.g. advanced HIV).
- A lumbar puncture may still be possible if the platelet count is 50 × 10⁹/L; Seek haematological advice.

Other Cerebrospinal fluid findings:

Measuring anti-HSV antibodies in the cerebrospinal fluid may be diagnostically useful, but detectable cerebrospinal fluid antibody levels usually develop after the first week of the illness. ⁽⁷¹⁾

There are several problems with the interpretations of serum and cerebrospinal fluid viral antibodies.⁽⁷²⁾

Rises in antiviral antibody titres may be non-specific and indicate polyclonal activation due to the infection. Also, raised antiviral antibodies in a single serum sample may reflect persistent viral antibody levels from previous infection, or reactivation rather than a primary infection.

More recently, diagnostic polymerase chain reaction (PCR) for viral DNA amplification technique has significantly facilitated the diagnosis of infective encephalitis. Cerebrospinal fluid polymerase chain reaction is diagnostic for encephalitis due to HSV, VZV, cytomegalovirus, and EBV.⁽⁷¹⁾

There are several advantages of the polymerase chain reaction technique. This technique is exquisitely sensitive for the presence of viral genome in spinal fluid, can be rapidly accomplished (within 6–8 hours), requires only a very small volume of cerebrospinal fluid, and is highly specific for certain viruses for example, HSV since the primers, if appropriately chosen, will not amplify DNA sequences from other viruses.⁽⁷¹⁾

(3) Virological tests in encephalitis

General

The gold standard of diagnosis in encephalitis is virus isolation in cell culture, now to be replaced by the detection of specific nucleic acid from CSF or brain.⁽⁷³⁻⁷⁶⁾ Intrathecal antibody production to a specific virus is similarly a strong evidence for aetiology.^(77,78) Virus detection from throat, stool, urine or blood as well as systemic serological responses like seroconversion or a specific IgM provides less strong evidence.^(79, 80)

The CSF is a convenient specimen and is recommended for neurological viral diagnosis in general.⁽⁸¹⁾ Brain biopsy is invasive and not used in routine clinical practice. At autopsy brain material will be obtained for virus isolation, nucleic acid and antigen detection as well as for immune histochemistry and in situ hybridization.

Viral culture

Viral cultures from CSF and brain tissue as well as from throat and stool specimens are performed in four different cell lines: African green monkey cells, Vero cells, human amniotic epithelial cells and human embryonic skin fibroblasts. Cells are evaluated daily for cytopathic effect and the findings are confirmed by a neutralizing or an immunofluorescence antibody test. Viral cultures from CSF are positive in young children with enteroviral infection but only seldom, in <5%, in other cases.^(82, 83)

Nucleic acid detection

For nucleic acid detection, polymerase chain reaction (PCR) technology provides the most convenient test. Assays for HSV-1, HSV-2, VZV, human herpesvirus 6 and 7, CMV, EBV, enteroviruses and respiratory viruses as well as for HIV can be performed from CSF samples or brain tissue.⁽²⁾

Serological tests

Antibodies to HSV-1, HSV-2, VZV, CMV, human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7), CMV, EBV, respiratory syncytial virus (RSV), HIV, adeno, influenza A and B, rota, coxsackie B5, non-typed entero and parainfluenza 1 viruses as well as *Mycoplasma pneumoniae* are measured from serum and CSF by using enzyme immunoassay (EIA) tests and antibodies for *Chlamydia pneumoniae* by microimmunofluorescence (MIF) test. ⁽⁸⁴⁻⁸⁸⁾

These tests are sensitive enough to detect even low amounts of antibodies from the CSF. The antibody levels in serum and CSF are compared with each other in the same dilution of 1:200. If the ratio of antibody levels is ≤ 20 , it indicates intrathecal antibody production within the brain provided that no other antibodies are present in the CSF, i.e. the blood–brain barrier (BBB) is not damaged. The presence of several antibodies in the CSF suggests BBB breakdown, while the presence of specific Immunoglobulin M (IgM) in the CSF indicates CNS disease. ⁽⁷⁹⁾

Antigen detection

Antigens of HSV, VZV and RSV, influenza A and B, parainfluenza 1 and 3, and adenoviruses can be studied from throat specimens with a conventional immunofluorescence (IF) test or with an EIA test and may provide a possible aetiology for encephalitis. ⁽²⁾

Neuroimaging.

Computed tomography (CT) and Magnetic resonance imaging (MRI) are most frequently used to evaluate patients with encephalitis, with MRI being more sensitive and specific. ⁽⁸⁹⁾

These studies may also be useful in excluding other conditions with a clinical presentation similar to that of encephalitis. CT (with and without intravenous contrast administration) should only be used if MRI is unavailable, impractical, or cannot be performed. Although MRI is useful for detection of early changes in encephalitis, it does not necessarily assist in differentiation of a specific etiology of encephalitis, and the findings may initially be normal or remain normal during the course of illness. Diffusion-weighted imaging is superior to conventional MRI for the detection of early signal abnormalities in viral encephalitis caused by herpes simplex virus, enterovirus 71, and West Nile virus. ⁽⁸⁹⁾

Some characteristic neuroimaging patterns have been observed in patients with encephalitis caused by specific agents. In patients with herpes simplex encephalitis, there may be significant edema and hemorrhage in the temporal lobes, as well as hypodense areas on T1-weighted images and non homogeneous contrast enhancement; bilateral temporal lobe involvement is nearly pathognomonic for herpes simplex encephalitis, but this is a late development. More than 90% of patients with herpes simplex encephalitis documented by CSF PCR will have abnormalities seen on MRI. ⁽⁹⁰⁾

In patients with encephalitis caused by flaviviruses and Eastern equine encephalitis virus, MRI may display a characteristic pattern of mixed intensity or hypodense lesions on T1-weighted images in the thalamus, basal ganglia, and midbrain; these lesions are

hyperintense on T2 and fluid-attenuated inversion recovery (FLAIR) images. In patients with enterovirus 71 encephalitis, MRI may demonstrate hyperintense T2 and FLAIR lesions localized to the midbrain, pons, and medulla. Follow-up MRI may also be useful for evaluation of evolving necrosis or demyelination. In cases of suspected ADEM, MRI is the diagnostic neuroimaging procedure of choice, generally revealing multiple focal or confluent areas of signal abnormality in the subcortical white matter and sometimes, subcortical gray matter on T2 and FLAIR sequences. ^(91, 92)

Other modalities

MR spectroscopy identifies and quantifies concentrations of various brain metabolites, and so may help distinguish normal from diseased brain tissue, and characterise the nature of the damage, particularly distinguishing inflammatory from neoplastic processes; however there are no prospective studies assessing its diagnostic role. Single photon emission computed tomography (SPECT) may show focal hypoperfusion persisting after recovery from acute viral encephalitis. ⁽⁹³⁾

However, it has been used mainly as a research tool and appears to have a little application in suspected acute encephalitis in practice. Fluorodeoxyglucose positron emission tomography (PET) shows abnormalities in acute viral encephalitis ^(93, 94) with regions of 18F-fluoro-2-deoxy-D-glucose FDG-PET hypermetabolism seen most frequently in the medial temporal lobes (sometimes reflecting seizure activity). However PET scanning is not practical or sufficiently informative to be used in children with suspected acute viral encephalitis.

The Electroencephalography (EEG) is abnormal in most patients with encephalopathy, including more than 80% of those with acute viral encephalitis. ⁽⁹⁵⁾ When patients have a more subtle presentation, it can be helpful in determining whether abnormal behavior is due to psychiatric causes or is an early feature of encephalopathies. EEG is also useful in determining whether an individual has non-convulsive or subtle clinical seizures, which occur in both HSV encephalitis and other encephalopathies. ^(96, 97)

In HSV encephalitis EEG abnormalities include non-specific diffuse high amplitude slow waves, sometimes with temporal lobe spike-and-wave activity and periodic lateralized epileptiform discharges (PLEDs). ⁽⁹⁸⁾ Even though PLEDs occur in many cases of HSV encephalitis ⁽⁹⁹⁾ and are at one stage considered pathognomonic, they are now recognized in other viral encephalitides ⁽¹⁰⁰⁾ and non-infectious conditions, and it is accepted that there are no EEG changes diagnostic of HSV encephalitis. ⁽¹⁰¹⁾

For example when PLEDs are identified in patients with a sub-acute or chronic encephalopathy this would be suggestive of SSPE. ^(102, 103)

Brain biopsy

Isolation of HSV from brain tissue obtained at biopsy was previously considered the gold standard for the diagnosis of HSE.

Brain biopsy was a part of all the major treatment trials of HSE conducted by the National Institutes of Allergy and Infectious Diseases Collaborative Antiviral Study Group (NINAIDCASG) in the 1980. ^(104, 105)

In these trials, 1 cm³ of the brain tissue was obtained from the anterior portion of the involved inferior temporal gyrus by subtemporal craniectomy under general anaesthesia. The sensitivity of the brain biopsy in HSE exceeds 95% with specificity greater than 99%. Brain biopsy in acute encephalitis was routinely advocated during the days when vidarabine was the only therapeutic agent in HSE. ⁽⁶²⁾

The introduction of acyclovir early in the treatment of HSE has largely rendered this policy unnecessary. Presently, brain biopsy in the setting of acute encephalitis may still have to be considered only if the diagnosis of HSE itself is doubtful. Brain biopsy in acute encephalitis may also be considered when surgical decompression is the treatment of choice for raised intracranial pressure refractory to medical management. ⁽⁶²⁾

Differential Diagnosis

The differential diagnosis of neurologic illness compatible with encephalitis is broad (see Table 4: differential diagnosis of neurologic illness compatible with encephalitis). In a patient with encephalopathy or possible encephalitis, a careful history and physical examination is the cornerstone of the evaluation. ⁽⁴⁹⁾

Table (4): Differential diagnosis of neurologic illness compatible with encephalitis.

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- 1) Metabolic diseases
 - 2) Toxic disorders
 - 3) Mass lesions
 - 4) Subarachnoid hemorrhage
 - 5) Embolic lesions
 - 6) Acute demyelinating disorders
 - 7) Status epilepticus
 - 8) Infectious diseases
 - 9) Postinfectious diseases
 - 10) Acute confusional migraine
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Treatment

Many patients with suspected acute encephalitis are critically ill. Their behaviour is often disturbed and they are at risk of seizures, malignant raised intracranial pressure, aspiration, systemic complications of infection, electrolyte disturbances, and death. Because it is a relatively rare condition, medical teams caring for patients with encephalitis often have limited experience with the condition. Patients require close monitoring in a quiet environment but do not routinely require isolation. Unlike stroke, where clear evidence exists to support patient management in specialist units, no such studies have been undertaken for encephalitis. ⁽¹⁰⁶⁾

Specific antiviral therapy should be initiated when appropriate. Vital functions, including respiration and blood pressure, should be monitored continuously and supported as required. In the initial stages of encephalitis, many patients will require care in an intensive care unit. ⁽¹⁰⁷⁾

Basic management and supportive therapy should include careful monitoring of intracranial pressure (ICP), fluid restriction, avoidance of hypotonic intravenous solutions, and suppression of fever. ⁽⁹⁹⁾

Seizures should be treated with standard anticonvulsant regimens, and prophylactic therapy should be considered in view of the high frequency of seizures in severe cases of encephalitis. As with all seriously ill, immobilized patients with altered levels of consciousness, encephalitis patients are at risk for aspiration pneumonia, stasis ulcers and decubiti, contractures, deep venous thrombosis and its complications, and infections of indwelling lines and catheters. ⁽¹⁰⁷⁾

Acyclovir is of benefit in the treatment of HSV and should be started empirically in patients with suspected viral encephalitis, especially if focal features are present, while awaiting viral diagnostic studies. Treatment should be discontinued in patients found not to have HSV encephalitis, with the possible exception of patients with severe encephalitis due to VZV or EBV. Adults should receive a dose of 10 mg/kg of acyclovir intravenously every 8 h (30 mg/kg per day total dose) for a minimum of 14 days. ⁽¹⁰⁷⁾

CSF PCR can be repeated at the completion of the 14-day course, with PCR-positive patients receiving an additional 7 days of treatment, followed by a repeat CSF PCR test. ⁽¹⁰⁷⁾

Neonatal HSV CNS infection is less responsive to acyclovir therapy than HSV encephalitis in adults; it is recommended that neonates with HSV encephalitis receive 20 mg/kg of acyclovir every 8 h (60 mg/kg per day total dose) for a minimum of 21 days.

Each dose should be infused slowly over 1 h rather than by rapid or bolus infusion, to minimize the risk of renal dysfunction. Care should be taken to avoid extravasation or intramuscular or subcutaneous administration. ⁽¹⁰⁷⁾

The alkaline pH of acyclovir can cause local inflammation and phlebitis (9%). Dose adjustment is required in patients with impaired renal glomerular filtration. Penetration into CSF is excellent, with average drug levels ~50% of serum levels.

Complications of therapy include elevations in blood urea nitrogen and creatinine levels (5%), thrombocytopenia (6%), gastrointestinal toxicity (nausea, vomiting, diarrhea) (7%), and neurotoxicity (lethargy or obtundation, disorientation, confusion, agitation, hallucinations, tremors, seizures) (1%).

Oral antiviral drugs with efficacy against HSV, VZV, and EBV, including acyclovir, famcyclovir, and valacyclovir, have not been evaluated in the treatment of encephalitis either as primary therapy or as supplemental therapy following completion of a course of parenteral acyclovir. ⁽¹⁰⁷⁾

In circumstances where ongoing intravenous treatment is proving difficult (for example, in a child who is now fully conscious), oral valacyclovir may be reasonable, ⁽¹⁰⁸⁾ although we would only consider this after the first 10 days of intravenous treatment. ⁽¹⁰⁸⁾

Valacyclovir is the valene ester of acyclovir, which is converted to acyclovir after absorption, and has good oral bioavailability. Although oral valacyclovir may have a role, oral acyclovir should not be used in HSV encephalitis, because the levels achieved in the CSF are inadequate. ⁽⁶³⁾

The duration of treatment is 21 days for immunosuppressed patients. Acyclovir is effective against encephalitis due to HSV-1, HSV-2, and VZV. ⁽¹⁰⁹⁾ Doses of acyclovir in VZV encephalitis are similar to HSE.

Reports have indicated that relapse after therapy may be as high as 5% but relapse has not been documented when higher doses were administered for 21 days. ⁽¹¹⁰⁾

Although acyclovir is a relatively safe drug it has important side effects. It is predominantly excreted by the kidneys, where it can cause renal impairment through crystalluria resulting in obstructive nephropathy. ⁽¹¹¹⁾

This reversible nephropathy usually manifests after 4 days of intravenous therapy and can affect up to 20% of patients. ^(112,113)

Corticosteroids

Large doses of corticosteroids (dexamethasone) as an adjunct treatment for acute viral encephalitis are not generally considered to be effective and their use is controversial. Probably the best evidence for steroid therapy is in VZV encephalitis. Primary VZV infection may cause severe encephalitis in immunocompetent children due to cerebral vasculitis. ⁽¹¹⁴⁾

Vasculitis following primary and secondary VZV infection is recognized to lead to a chronic course in immunocompetent children and adults (granulomatous angiitis). HSE is occasionally complicated by severe, vasogenic cerebral oedema with CT or MRI evidence of midline shift where high dose steroids may have a role. ⁽¹¹⁵⁾

Steroid pulse therapy with methylprednisolone has been observed to be beneficial in a small number of patients with acute viral encephalitis who had progressive disturbance of consciousness, an important prognostic factor for outcome. ⁽¹¹⁵⁾

Based on available data, combined acyclovir/steroid treatment may be advised in immunocompetent individuals with severe VZV encephalitis and probably in other cases of acute viral encephalitis where progressive cerebral oedema documented by CT/MRI complicates the course of illness in the early phase.

High dose dexamethasone or pulse methylprednisolone are both suitable agents. The duration of steroid treatment should be short (between 3 and 5 days) in order to minimize adverse effects (e.g. gastrointestinal haemorrhage, secondary fever and infections). ⁽²⁾

Other treatments to be considered

In some circumstances, both antiviral and immunosuppressive drugs are given. For example, in HSV encephalitis corticosteroids are sometimes used in addition to acyclovir as described above. In VZV encephalitis corticosteroids are used alongside acyclovir because of the strong vasculitic component of the disease.

Severe CMV and HHV-6 infections are treated with gancyclovir, foscarnet or cidofovir, severe adenovirus infections have been treated with cidofovir or ribavirin and Pleconaril has been used for severe enterovirus infections, particularly in the immunocompromised, although its overall role remains unclear. ^(116,117)

Interferon alpha has been used in West Nile virus and other flavivirus infections, but a randomised controlled trial in Japanese encephalitis showed it was not effective. ⁽¹¹⁸⁾

In patients with brain swelling, corticosteroids and mannitol are often used to control raised intracranial pressure. A recent trial suggests steroids may be beneficial even in patients without marked swelling. ⁽¹¹⁹⁾ Their role in HSV encephalitis merits further study. ⁽¹²⁰⁾

In patients with severe brain swelling, decompressive hemicraniectomy is sometimes performed. Antibiotic treatment with a cephalosporin is often also given, especially if the initial CSF findings could be consistent with bacterial disease. If listeria is suspected, ampicillin and gentamicin, or high dose cotrimoxazole, should be given. ⁽⁶³⁾

Antibiotic treatment with a cephalosporin is usually given in children until a proven viral cause is identified. It is wise to treat until the blood and CSF cultures are known to be negative.

Management of seizures, raised intracranial pressure and other complications

Seizures are common in encephalitis, particularly in children. In adults, seizures can be useful in distinguishing acute viral encephalitis from para-infectious inflammatory encephalopathies. ⁽¹²¹⁾

Uncontrolled seizures lead to raised intracranial pressure, increased metabolic activity, acidosis and vasodilatation, which in turn leads to further raised pressure. The resulting positive feedback cycle can ultimately precipitate brain shift and herniation. If seizures are not easily controlled with phenytoin and low doses of benzodiazepines, patients should be intubated and ventilated mechanically, so that higher doses of sedating anticonvulsive drugs, including benzodiazepines and phenobarbital, can be used. Electroencephalographic monitoring, with or without continuous function and analysing monitoring (CFAM), should be used to detect ongoing epileptic activity. ⁽¹²¹⁾

Standard measures to control raised intracranial pressure include nursing the patient at 30° head up, keeping the head straight to ensure there is no obstruction to venous return, and ventilating to maintain a low arterial partial pressure of carbon dioxide (PCO₂). Although there are no good data for viral encephalitis, data from other infectious encephalopathies suggest that osmotic diuretics produce a short-term reduction in pressure. ⁽¹²¹⁾

Status epilepticus caused by encephalitis should be treated vigorously using a structured protocol to ensure optimal control. ^(122,123) The current standard initial therapy is intravenous lorazepam, 0.1 to 0.2 mg/kg, up to 4 mg maximum. Seizures associated with encephalitis may be refractory to the usual therapy, and other anticonvulsants may be required to achieve and maintain control of seizures. In patients who fail initial therapy and are in medically refractory status epilepticus, continuous EEG monitoring usually is

recommended to monitor the efficacy of the therapy, especially when the patient is in non convulsive status epilepticus. ^(124,125)

If after a second attempt, lorazepam fails to control the seizures, intravenous phenytoin (preferably fosphenytoin in children) is the next drug of choice. The dose is 18 to 20 mg/kg, maximum 1000 mg, given over 20 minutes. Fosphenytoin is preferred because it can be administered faster and does not cause sclerosis of the veins as does phenytoin and is not as likely to cause cardiac arrhythmias. Virtually all patients who require therapy beyond lorazepam need to be intubated to prevent respiratory embarrassment. If fosphenytoin is unsuccessful, or as an alternative to fosphenytoin, intravenous midazolam has gained favor in recent years. ^(126,127)

The initial dose is 0.1 to 0.2 mg/kg over 5 minutes, with a maintenance infusion starting at 0.05 mg/ kg/hr up to a maximum of 0.4 mg/kg/hr. Another alternative therapy is propofol, which generally is administered by an anesthesiologist.

Bed sores are a risk in immobile patients, and appropriate mattresses and regular turning are needed. Patients with encephalitis are also at risk of secondary pneumonia, due to aspiration, and urinary tract infections. Passive and active limb movements will reduce the risk of limb contractures, which can occur in patients with limb weakness.

Management in the recovery period

Ideally, a full neuropsychological assessment should be organised at hospital discharge, or soon after. This should include cognitive function, intelligence, memory and speech assessment, because these help determine the extent of any damage, and the help that might be needed. Regular out-patient assessment following encephalitis is especially important in children. Behavioural and psychiatric disturbances are common and may include depression or disinhibition.

Antidepressants and mild night-time sedatives may be necessary. Other disabilities in the recovery period or afterwards include seizures, post-encephalitic parkinsonism seen after encephalitis lethargica, and encephalitis caused by flaviviruses. The risk of seizures is greatest in those who had seizures during the acute period; in one study the cumulative risk of seizures at 5 years was 10% for patients with no acute seizures, which increased to 20% for those with acute seizures. ⁽¹²⁸⁾

Memory difficulties can be particularly prominent after HSV encephalitis. A range of practical approaches can help to overcome these difficulties such as the patient keeping a notebook and diary, labelling items around the house, and leaving messages as reminders. More sophisticated aids being developed include a neuropage system, which sends pager reminder messages throughout the day, and a camera, worn around the neck, which automatically takes pictures throughout the day as a reminder of what the patient has been doing (Sense Cam). Excellent help and advice can be obtained from patient support groups, such as the encephalitis society.

Rehabilitation

The sequelae and consequences of encephalitis may not be immediately apparent when a patient is discharged from hospital following the acute illness. However, anxiety, depression and obsessive behaviours often become evident subsequently, and may be more frequently encountered after encephalitis than other causes of acute brain injury.⁽¹²⁹⁾

A charity-commissioned study of encephalitis patients found that 33% were discharged without outpatient follow-up although 96% reported ongoing complications from their illness.⁽¹³⁰⁾

A broad and comprehensive approach to both assessment and rehabilitation is necessary, with input from specialists in neuropsychology and neuropsychiatry as central components,⁽¹³¹⁾ in addition to speech and language therapy, neuro-physiotherapy, and occupational therapy. Access to specialist brain injury rehabilitation services is key to recovery in many cases,^(130,132) and patients and their families greatly value the support provided by voluntary sector organisations such as the Encephalitis Society.

Patients affected by encephalitis and those supporting them require information on the condition and its consequences, and directions on how to access this information.⁽⁶³⁾

In one survey one-third of patients were discharged from hospital without their families recalling being informed of the diagnosis.⁽¹³²⁾

Information and support reduces isolation, helps family adjustment and can provide useful signposting to other services as appropriate.⁽¹³⁰⁾

Complications

Neuropsychological and Psychological Outcomes

Complications of viral encephalitis can include motor deficits, aphasia, amnesia, global cognitive decline, and epilepsy.⁽¹³³⁾ Due to the effects of the virus in the temporal lobes, the most commonly reported neuropsychological impairments, which may occur together or in isolation, are anterograde and retrograde amnesia^(129,133) The retrograde amnesia can include impairments in semantic or episodic memory and in the ability to recall names, faces, and episodes involving familiar or famous people. Less widespread damage to the temporal lobes and fronto-basal areas may result in less severe amnesic disorders.⁽¹³³⁾ While not typical in HSE, isolated amnesia or category specific anomia has been reported.⁽¹³³⁾

In addition to aphasia, anomia (i.e., encompassing visual identification, drawing, or sorting of pictures which may suggest widespread loss of semantic knowledge), executive dysfunction (e.g., the stroop task, letter fluency, card sorting tasks and the Cognitive Estimations Test), and impairments in visual perception^(129,133) have also been reported. Cognitive impairments may remain stable or diminish over time. However, some HSE patients may show a gradual, global cognitive deterioration.⁽¹³³⁾ Another common sequel of encephalitis is epilepsy, which when accompanied by frequent seizure activity may have a detrimental effect on cognitive functioning, with memory performance particularly vulnerable due to neuronal loss in hippocampal regions.⁽¹³³⁾

In general, the cognitive deficits in non-HSE encephalitis are less frequent as well as less severe than those in HSE.⁽¹³³⁾ However, while other forms of encephalitis have received less thorough neuropsychological investigation, cognitive impairments have been reported following herpes simplex virus type 2, St Louis encephalitis, influenza virus types A and B, and Japanese encephalitis.⁽¹²⁹⁾

Psychological

The patient's mental state is frequently confused and disoriented with neuropsychiatric symptoms that may include agitation, emotional lability, behavioral disorders, personality changes, hallucinations, and frank psychosis.⁽⁶⁾ In HSE, psychiatric and behavioral symptoms have been reported to precede, accompany, and follow the acute illness.⁽¹³³⁾ In some patients, the altered behavior and psychotic episodes are first observed long before the onset of neurological symptoms, which may delay the process of arriving at the correct diagnosis.⁽¹³³⁾

Following treatment with acyclovir, 40–60% of the HSE survivors demonstrate persistent personality and behavioral abnormalities and instability, but the emotional symptoms appear to be milder.⁽¹³³⁾ Common symptoms include panic and anxiety disorders, phobic anxiety, major affective disorders (e.g., bipolar), manic behavior, aggressive outbursts, irritability, depression, and obsessive-compulsive behaviors.^(129,133) Single-case studies have reported more severe cases with schizophrenic form disorder.⁽¹²⁹⁾ The neuropsychiatric symptoms arising from limbic lobe involvement are well-known and can include emotional or mood disorders (e.g., rage, aggression, depression and mania), delusions, hallucinations, anxiety and dissociative disorders as well as sexuality changes, and hyperoral behaviors.⁽¹³³⁾

Prognosis and outcome

In patients with herpes simplex encephalitis, predictors of an adverse outcome include age of the patient (130 years), level of consciousness (Glasgow coma score, 6), and duration of symptoms prior to starting acyclovir therapy (14 days)⁽¹¹⁰⁾; mortality decreased to 8% if therapy was initiated 4 days after onset of clinical symptoms.⁽¹³⁴⁾

Although treatment with acyclovir has reduced the mortality of HSV, the morbidity remains high.⁽¹⁰⁾ Poor prognostic factors after HSV encephalitis include age above 60, reduced coma score on admission,⁽¹³⁵⁾ and delays between hospitalization and starting acyclovir treatment (especially delays of more than two days).⁽¹³⁴⁾

Two thirds of survivors have significant neuropsychiatric sequelae, including memory impairment (69%), personality and behavioural change (45%), dysphasia (41%) and epilepsy in up to 25%.⁽¹³⁴⁾

Prevention

The widespread use of effective attenuated viral vaccines for measles, mumps, and rubella almost has eliminated CNS complications from these diseases in the United States. The control of encephalitis caused by arboviruses has been less successful because specific vaccines for the arbovirus diseases that occur in North America are unavailable. Control of insect vectors by suitable spraying methods and eradication of insect breeding sites is useful.⁽⁴⁹⁾

Neopterin

Definition:

Neopterin is a marker associated with the activity of monocytes, macrophages, which is produced and released into body fluids, under stimulation of T-lymphocytes. Neopterin is released into body fluids and participate in immune response and can be used to monitor activation of cell mediated immunity. ^(136, 137)

1. Biochemistry of Neopterin:

Neopterin is 2-amino-4-hydroxy-6- (D-erythro-1', 2', 3' trihydroxypropyl) pteridine. Because of its chemical structure, it belongs to the group of pteridines commonly found in living cells. Pteridines can be divided into aromatic pteridines, 7, 8- dihydroneopterines, 5, 6, 7, 8-tetrahydrobiopterines, lumazines and miscellaneous pteridines. According to this classification, neopterin is an aromatic pteridine with a low molecular mass (253 Da). ⁽¹³⁸⁾ (see figure 1) ⁽¹³⁸⁾

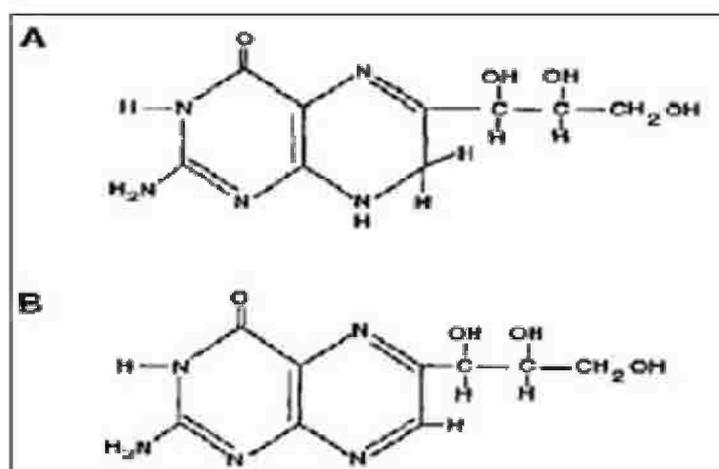


Figure (1): Structure of (A) 7, 8-dihydroneopterin and (B) Neopterin. ⁽¹³⁸⁾

2. Synthesis of neopterin:

Substrate for the biosynthesis of neopterin is guanosine 5'- triphosphate (GTP) which is found in human monocyte-derived macrophages and dendritic cells. GTP is cleaved by GTP cyclohydrolase I to 7, 8-dihydroneopterin triphosphate. The GTP-cyclohydrolase I is triggered by soluble mediators derived from activated T-lymphocytes. ⁽¹³⁹⁾

Type1 T helper (Th1- type) cytokine Interferon gamma IFN- γ exhibited the strongest effect on neopterin release especially when co-stimulated by other cytokines, as tumor necrosis factor- α (TNF- α) or lipopolysaccharides (LPS). At higher concentrations interferon-alpha (INF- α) and interferon beta (INF- β) established a similar effect in monocyte-derived macrophages. In contrast, in human monocyte-derived dendritic cells, IFN- α , β and γ had similar capacity to induce formation of neopterin. ⁽¹⁴⁰⁾

Under resting condition, the major pathway for the metabolism of 7, 8-dihydroneopterin triphosphate inside the human macrophages and dendritic cells is directed toward the formation of 5, 6, 7, 8-tetrahydrobiopterin by the enzyme pyrovoyltetrahydropterin synthase (PTPS) and only a minor amount is directed toward the formation of 7, 8- dihydroneopterin by the phosphatase enzyme with subsequent oxidation in to neopterin. However during immune activation; PTPS shows low activity, hence the metabolism of 7, 8-dihydroneopterin is directed mainly toward the production of large amount of neopterin at the expense of biopterin derivatives.⁽¹⁴⁰⁾ See figure (2).⁽¹⁴¹⁾

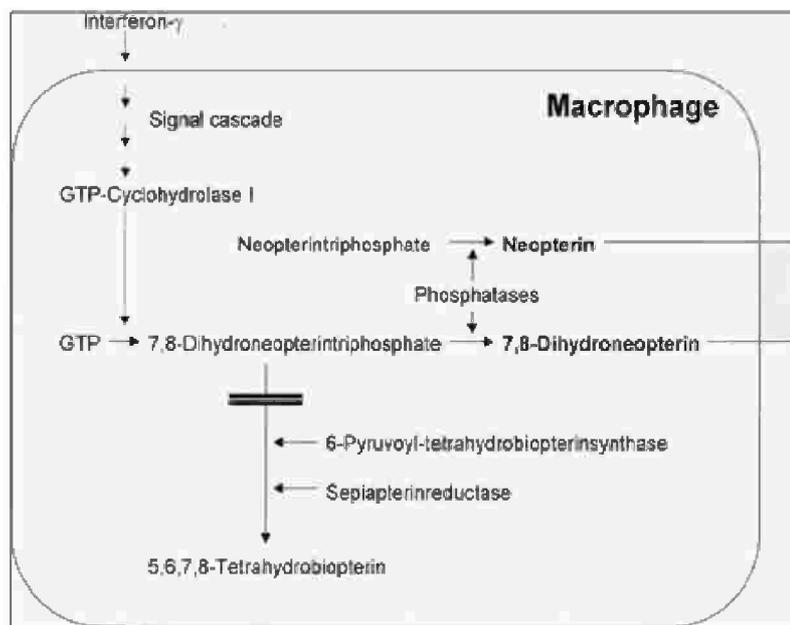


Figure (2): Biosynthesis of Neopterin derivatives in human monocytes/macrophages.⁽¹⁴¹⁾

(3) Body Distribution and Elimination of Neopterin:

Neopterin is only detectable in body fluids such as serum, urine, cerebrospinal fluid (CSF), synovial fluid, pancreatic juice, ascetic fluid and oral fluids. The half-life of neopterin within the circulatory system was calculated to be approximately 90 min. The neopterin: 7, 8-dihydroneopterin ratio of 1:2 was formed to be nearly constant suggesting that neopterin is not further metabolized after production. Renal excretion represents the only way of neopterin elimination from the circulation⁽¹³⁹⁾

(4) Factors Affecting Neopterin Concentration:

Age and Gender:

Blood neopterin concentrations are age-dependent. In fetal serum obtained by cordocentesis, neopterin levels increase with the gestational age, peaking in the late third trimester.⁽¹⁴²⁾ Significantly high serum neopterin concentrations were also observed with older age.⁽¹⁴³⁾ Serum neopterin levels are not related to gender.⁽¹⁴⁴⁾

Pregnancy:

In the course of normal pregnancy, maternal serum and urinary neopterin show a gradual increase to reach their peaks in the third trimester. Normalization of maternal neopterin is usually expected 6-12 months after delivery.⁽¹⁴⁵⁾

Others:

Neopterin level increase in normal persons with increased age and in white race and decrease in smokers compared to non-smokers.⁽¹⁴⁶⁾ It was found that factors like body mass index is associated with increase serum neopterin concentrations, whereas diastolic blood pressure and the number of daily smoked cigarettes are associated with decrease neopterin concentrations. The suppressive effect of tobacco smoke on the human immune system could explain the lower neopterin production of smokers.⁽¹⁴⁷⁾

Elevated neopterin levels was found with viral infections,^(148,149) immune system activation, autoimmune disorders,⁽¹⁵⁰⁾ malignant disease,⁽¹⁵¹⁾ allograft rejection⁽¹⁵²⁾ cardiac and renal failure and coronary artery Disease.⁽¹⁵³⁾ Increased serum and urinary levels can be of clinical value in the diagnosis and prognosis of conditions associated with cell-mediated immunity.⁽¹⁵⁴⁾

(4) Physiological Role of Neopterin:

The specific nature of neopterin biosynthesis in human monocyte-derived macrophages and dendritic cells signifies that neopterin is a marker for an activated Th1 mediated cellular immune system.⁽¹⁵⁵⁾ Cell-mediated immunity is an immune response that does not involve antibodies or complement production but rather involves the activation of macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.⁽¹⁵⁶⁾

Cellular immunity protects the body by activating antigen specific cytotoxic T-lymphocytes that are able to induce apoptosis in body cells displaying epitopes of foreign antigen on their surface such as (virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens), activating macrophages and natural killer cells, enabling them to destroy intracellular pathogens and stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.⁽¹⁵⁶⁾

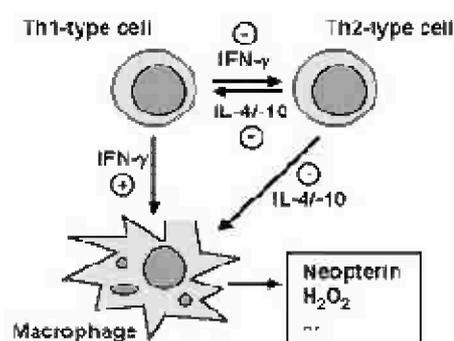


Figure (3): Cross regulatory influence between Th1 and Th2 lymphocytes.⁽¹⁵⁷⁾

Neopterin is not only a marker for an activated Th1 mediated cellular immune system but it acts as an amplifier of cell mediated response through the stimulation of TNF- α gene expression, the induction of TNF- α secretion by peripheral blood mononuclear cells and amplification of IFN- γ and IL-2 secretion. Moreover, neopterin exerts biochemical and physiological functions in the course of host defense reactions.⁽¹⁵⁵⁾

(5) Clinical Significance of Neopterin:

Increased production of neopterin in body fluids can be used to monitor activation of cell mediated immunity. High neopterin concentration in serum and urine were shown to be a reliable indicator for the severity of infectious, immunological and inflammatory disorders.⁽¹⁵⁸⁾ Neopterin measurement can help in predicting disease progression.⁽¹⁵⁹⁾

The most important clinical applications for the determination of neopterin are prognostic indicator and follow-up of chronic infections, monitoring of immune-stimulatory therapy, differential diagnosis of acute viral and bacterial infections, and early indications of complications in allograft recipients.⁽¹⁶⁰⁾

The release of neopterin begins 3 days before T cells proliferation reaches maximum, and an increase in neopterin production can be observed about one week before the appearance of specific antibodies. Therefore, neopterin may be of clinical use as an early inflammatory marker.⁽¹⁶¹⁾

Neopterin in viral infections:

Acute viral infections strongly increased neopterin production which in turn correlates with the activity of the disease. This was shown in acute viral hepatitis, Epstein-Barr virus, cytomegalovirus, measles, mumps, chicken pox, rubella and influenza viruses. Elevated neopterin levels in body fluids may be found at the end of the incubation period before the onset of clinical symptoms. The neopterin levels become detectable about two to four weeks before the rise of the specific anti-viral antibodies. During chronic viral infections such as infection by human immunodeficiency virus (HIV), neopterin production declines but does not normalize. On seroconversion, neopterin concentrations decline and normalize if the immune system successfully competes the virus.⁽¹⁶⁰⁾

High neopterin levels in CSF was detected in encephalitis of viral and bacterial origin.⁽¹⁶²⁾ In almost all patients with acute viral infections neopterin levels are increased. The elevated neopterin levels in body fluids may be found at the end of the incubation period before onset of clinical symptoms and before specific antibodies against the virus become detectable.^(163,164)

S 100 B Protein

Definition

S100B is a calcium-binding peptide produced mainly by astrocytes that exerts paracrine and autocrine effects on neurons and glia. In recent years, increased S100B in biological fluids has been shown to be a marker of brain damage both in adults and during the antenatal and postnatal periods. ⁽¹⁶⁵⁻¹⁶⁷⁾

Biochemistry

The S100 protein family is the largest subgroup of the calcium-binding EF-hand (helix E-loop-helix F) protein group. ⁽¹⁶⁸⁾ These were first described by Moore in 1965, and the name "S100" was given because of their solubility in a 100% saturated solution with ammonium sulphate. An unfractionated mixture of S100A1 and S100B was the first identified member of this family. ⁽¹⁶⁹⁾

At present, at least 25 proteins have been identified as belonging to the S100 protein family. ⁽¹⁷⁰⁾ S100 proteins belong to the S100 /calmodulin /parvalbumin/troponin C superfamily, with low molecular weight of about 9-13 kDa. ⁽¹⁶⁹⁾ Although they are usually thought to be calcium sensor proteins that modulate biological activities via calcium, ⁽¹⁷¹⁾ it is understood that some S100 proteins bind to Zinc (Zn⁺²) and calcium .

This binding is thought to suggest the possibility that their biological activity might be regulated by Zn⁺² and calcium. Their important property is that they can only be found in vertebrates and, unlike other EF hand proteins such as calmodulin and troponin C, S100 proteins form both homo and heterodimer protein complexes. ⁽¹⁷²⁾

Calcium is an intracellular second messenger that has several regulatory roles in many functional events and processes like conduction and transmission of the nerve impulse, muscle contraction, cell motility, growth and differentiation, gene expression, apoptosis, and necrosis. ⁽¹⁷³⁾ As a result of cellular evolution, calcium-binding proteins are formed to regulate the level of cytosolic calcium and transduce calcium signals. ⁽¹⁷⁴⁾

S100 protein contains a mixture of hetero- and homodimers of two types of subunit (α , β) with different amino acid compositions. S100A is described as a heterodimer of $\alpha\beta$, while S100B is a homodimer of $\beta\beta$. While S100 α is found abundantly in neurons in muscles, kidney and other organs, S100 β is localized in neural glial and Schwann cells. ⁽¹⁷⁵⁾

The protein previously known as S100 α is now known as protein S100A1, and the former protein S100 β is now named protein S100B. ⁽¹⁷⁶⁾

Body distribution and elimination:

S100B is located in the cytoplasm and nucleus of the astrocytes along with other members of the S100 family, and it regulates the cytoskeletal structure and cell proliferation. ⁽¹⁷⁷⁾

Although it has been shown that S100B is mainly found in astroglial and Schwann cells, it has also been found in adipocytes, chondrocytes, lymphocytes, bone marrow cells, and melanocytes.⁽¹⁷⁸⁾ S100B is mainly eliminated by the kidney.⁽¹⁶⁸⁾

S100 B as a Marker in Neurologic Disorders:

As such a biomarker, S100B is primarily produced by astrocytes in the CNS and represents astrocytic activation. An immunohistochemical study indicated that astrocytes are the predominant S100B-positive cells in gray matter, and oligodendrocytes are the predominant S100B-positive cells in white matter.⁽¹⁷⁹⁾ Intra- or extracellular S100B messenger ribonucleic acid (mRNA) and protein levels have been used as a parameter of astrocyte activation and/or death in several situations of brain injury.⁽¹⁷⁷⁾

Elevation in serum or CSF S100B concentrations is associated with a variety of disorders affecting the CNS. Although in many instances its release may be an effect of the condition rather than the cause, it is nonetheless strongly implicated that S100B can be considered a strong candidate as a marker of CNS injury.⁽¹⁸⁰⁾

S100B is not only implicated in the regulation of intracellular processes, but, it is also a secretory protein and exhibits cytokine-like activities, which mediate the interactions among glial cells and between glial cells and neurones. Interaction of S100B with the receptor for advanced glycation end products (RAGE), a multiligand receptor that has been shown to transduce inflammatory stimuli and effects of several neurotrophic and neurotoxic factors. Secretion of S100B from astrocytes is stimulated under metabolic stress (oxygen, serum and glucose deprivation) and is suppressed by glutamate. S100B acts in a dose-dependent manner: Nanomolar levels stimulate neurite growth and promote neurone survival.

Micromolar levels result in opposite effects and can even induce neuronal apoptosis, leading to the induction of pro-inflammatory cytokines such as interleukin1 β (IL- 1 β) or tumour necrosis factor α (TNF- α), and inflammatory stress-related enzymes such as inducible nitric oxide synthase (iNOS).^(181,182)

Astrocytes are the most common cells in brain tissue and S-100B is synthesized in astroglial and Schwann cells. It is thought to be a mediator of neuronal-glial interactions in normal brain.⁽¹³⁴⁾ Extracellular S100B might participate in brain inflammation by activating astrocytes, microglia and neurons.⁽¹⁷⁸⁾ Damage to the astrocytes causes leakage of S-100B protein extracellularly into the CSF and a small proportion leaks into the circulation.⁽¹⁸³⁾

So, the identification of S 100 B protein for brain damage in viral encephalitis could be useful.⁽¹³⁴⁾