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VIRAL MENINGOENCEPHALITIS:

PISA 2009-2015

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Table of contents

List of abbreviations

AIDS: Acquired Immunodeficiency Syndrome

BBB: blood brain barrier

CDC: Centers for Diseases Control and Prevention

CMV: cytomegalovirus

CNS: central nervous system

CRP: C-reactive protein

CT: computerized tomography

FDA: Food and Drug Administration

HHV-6: Human Herpes Virus 6

HIV: Human Immunodeficiency Virus

HSV: Herpes Simplex Virus

LCMV: Lymphocytic Choriomeningitis Virus

LP: lumbar puncture

MMR: measles, mumps and rubella

MRI: magnetic resonance imaging

PCR: polymerase chain reaction

PMN: polymorphonuclear cell

RT-PCR: real time PCR

SIADH: syndrome of inappropriate antidiuretic hormone secretion

SLE: St Louis Encephalitis

TOSV: Toscana Virus

VZV: Varicella Zoster Virus

WBC: white blood cell

WHO: World Health Organization

Abstract

Viral infections of the central nervous system (CNS) are underdiagnosed and their etiology often unknown.

Patients with a diagnosis of viral CNS infection were studied, including risk factors, epidemiological, clinical, laboratory and radiological criteria. We aimed to evaluate if timing of lumbar puncture and different cerebrospinal fluid characteristics were associated with a higher index of etiological diagnosis. A check list of serologic and molecular virology tests to be performed on blood and cerebrospinal fluid was developed.

The study was conducted during the 2009-2015 period.

Overall, 68 cases have been diagnosed. Males were 40/68 (55.7%), mean age was 49.6 (range 18-85). Etiology was established in 34/68 (50.0%) as follows: VZV 9 cases, Toscana Virus 7, HSV-1 6, Enterovirus 5, HSV-2 3, West Nile 2, HHV-7 and parvovirus B19 one.

When lumbar puncture was performed within 72h from the onset of symptoms, an etiologic diagnosis was made in 17/30 cases (56.6%); when lumbar puncture was performed after 72h from the onset of symptoms, an etiologic diagnosis was made in 11/32 cases (34.3%).

At CSF examination, leukocytes count was increased ($>5x10^9$/L) in 65/67 (97.0%) and lymphocytes were prevalent in 63/67 (94.0%); glucose was reduced (<50 mg/dl) in 12/67 (17.9%), but CSF/blood glucose ratio was less than 40% only in 2/67 (2.9%); proteins were increased (>60 mg/dl) in 45/67 (67.1%). Overall mean CSF leukocytes count was $285x10^9$/L while mean leukocytes count of Enterovirus infections was $152x10^9$/L and that of VZV infections was $625x10^9$/L. Mean protein concentration was 106.2 mg/dl while that of VZV infections was 237.9 mg/dl.

Additionally, a seroprevalence survey for Toscana Virus was conducted on consecutive patients admitted in the Infectious Diseases Unit, regardless the symptoms: IgG or IgM were positive in 35/100 cases (35%), with a clear trend to increase with age.

Introduction

An overwhelming number of people are affected by different viral infections every day. Clinically relevant involvement of the central nervous system (CNS) by viruses is a relatively uncommon event in the setting of these viral infections, but still represents a significant cause of morbidity and mortality, especially among infants and immunocompromised hosts, and a phenomenon greatly underestimated and unknown.

Possible causes of meningitis include bacteria, viruses, fungi, parasites, autoimmune diseases and medications. Viral infections of the CNS are probably the leading cause of meningitis but differentiating between viral and bacterial meningitis, presents still a considerable challenge, which aims to reduce antimicrobial resistance and hospital costs.

Viral infections of the CNS include different clinical features, most commonly encephalitis, meningitis and myelitis. However these are often concomitant and thus cannot be easily distinguished based on clinical findings alone.

Other less established and more unusual clinical features include progressive neurologic disorders, such as post-infectious encephalomyelitis in the setting of measles or Nipah virus encephalitis, and postpoliomyelitis syndrome occurring years after acute poliovirus infection.

The spectrum of possible involvement depends on the specific viral agent, host immune status, genetic asset and environmental factors. Examples of these factors include viral agents such as VZV which involve very often the brain parenchyma rather than the meninges alone, associated morbidity and mortality in immunocompromised patients or viral epidemics in specific periods of the year.

Furthermore, vector-transmitted viral infections causing a wide range of manifestations, including CNS involvement, in recent years have been responsible of epidemics in areas where they had never been present or had disappeared since decades (emerging and re-emerging infections).

Etiology and epidemiology

In the United States, more than 10,000 cases of viral meningitis are reported annually, but the actual incidence may be as high as 75,000. Lack of reporting is due to the uneventful clinical outcome of most cases and the inability of some viral agents to grow in culture. According to reports from the Centers for Disease Control and Prevention (CDC), inpatient hospitalizations resulting from viral meningitis range from 25,000-50,000 each year. An incidence of 11 new cases per 100,000 people per year has been estimated in some reports. [1]

The CDC estimates an annual incidence of approximately 20,000 new cases of encephalitis in the United States. The annual incidence of viral encephalitis is most likely underestimated, especially in developing countries, because of problems with pathogen detection.

As a consequence of mumps, measles, and rubella vaccination, enteroviruses have supplanted mumps as the most common cause of viral meningitis in children.[2,3]

Enteroviruses are said to account for 80% of cases in adults, but a wider range of causes is increasingly implicated.[4,5]

Worldwide, rabies and Japanese encephalitis virus (JEV) are responsible for an estimated annual mortality of 60,000 and 17,000 people, respectively. Cases of JEV encephalitis are restricted to Asia, whereas rabies is a scourge throughout Southeast Asia, Africa and Latin America and occurs, although less frequently, in other areas worldwide. [6]

Enteroviruses

Enteroviruses account for the majority of cases of viral meningitis in many case histories. Human enteroviruses cause a wide variety of diseases including polio, hand, foot and mouth disease, myocarditis, and aseptic meningitis, most commonly affecting and transmitted through children. [7–10]

They are members of the *Picornaviridae* family ("pico" for small, "rna" for ribonucleic acid).

Based on their antigenic properties, the original 64 HEV serotypes were initially grouped into polioviruses (PV), coxsackieviruses A (CA), coxsackieviruses B (CB), echoviruses (E), and the more recently identified enteroviruses (EV) 68 to 71. A new HEV classification based on molecular and biological data has recently been proposed as an alternative to the antigenic classification. This classification groups enteroviruses into five species: (1) *PV*, including poliovirus types 1, 2 and 3; (2) *HEV-A*, including 11 coxsackieviruses A and EV71; (3) *HEV-B*, including all coxsackieviruses B, all echoviruses, EV69 and CA9; (4)

HEV-C, including 11 other coxsackieviruses A; and (5) *HEV-D*, including EV68 and EV70. The enteroviruses previously classified as E22 and E23, which were shown to group independently, now constitute a new genus, *Parechovirus* (with two serotypes), in the family *Picornaviridae*. [11]

Coxsackie and echovirus are the most common causes of viral meningitis. However, other viruses such as poliovirus and enterovirus 71 (EV71) have also been demonstrated to have the ability to evoke aseptic meningitis. [12] Coxsackievirus B subgroups alone account for more than 60% of meningitis cases in children younger than age 3 months.

Enteroviruses are ubiquitous in the summer and early fall; their propensity to cause infection during the warmer months is the major factor in the higher incidence of aseptic meningitis during that time.

They enter the human host usually via the oral-fecal route, but can also spread through the respiratory route. The incubation period of non-polio infections is between 3–7 days and that of polio 1-3 weeks and the associated clinical findings may include pharyngitis, pleurodynia, rash, and pericarditis.

Expectant mothers infected with coxsackievirus B may have few symptoms, but their infants can acquire the infection perinatally and develop a potentially fatal illness, with the infection targeting mainly the heart.

Enteroviruses 70 and 71, which exhibit strong neurotropism, are associated with meningoencephalitis, polio-like paralytic syndromes, and Guillain-Barre syndrome, as well as aseptic meningitis.

The typical presentation of enteroviral meningitis includes headache, nausea, vomiting and photophobia.

Arboviruses

Arboviruses account for about 5% of cases of meningitis in North America.

Arboviruses consist of more than 500 viruses from different viral families, all given the common name "ar-bo" for arthropod-borne virus (table 1).

Table 1. Classification of main arbovirus pathogens for humans

Family	Genus	Main species	vector	Clinical manifestations
Togaviridae	Alphavirus	Western Equine Encephalitis	Mosquito	Encephalomyelitis
		Easernt Equine Encephalitis	Mosquito	Encephalomyelitis
		Venezuelan Equine Encephalitis	Mosquito	Encephalomyelitis
		Sindbis	Mosquito	Fever, exanthema
		Chikungunya	Mosquito	Fever, exanthema
		O'nyong-nyong	Mosquito	Fever, exanthema
		Mayaro	Mosquito	Fever, exanthema
		Ross River	Mosquito	Fever, exanthema, arthritis
		Semliki Forrest	Mosquito	Fever, exanthema, arthritis
Flaviridae	Flavivirus	St Louis Encephaltis	Mosquito	Encephalitis
		Japanese Encephalitis	Mosquito	Encephalitis
		Murray Valley Encephalitis	Mosquito	Encephalitis
		Rocio	Mosquito	Encephalitis
		West Nile	Mosquito	Encephalomyelitis, meningitis, exanthema
		Dengue	Mosquito	Fever, hemorrhagic fever
		Yellow Fever	Mosquito	Hepatitis, hemorrhagic fever
		Tick Born Encephalitis	Tick	Encephalitis
		Omsk Hemorrhagic Fever	Tick	Hemorrhagic fever
		Kyasanur Forrest	Tick	Hemorrhagic fever
		Powassan	Tick	Encephalitis
Bunyaviridae	Orthobunyavirs	Bunyamwera	Mosquito	Hemorrhagic fever
		Californian Encephalitis	Mosquito	Encephalitis
		La Crosse	Mosquito	Encephalitis
		Simbu	Mosquito	Encephalitis
	Nairovirus	Crimean Congo Hemorrhagic Fever	Tick	Hemorrhagic fever
	Phlebovirus	Phlebotomus Fever (various species)	Phlebotomus	Fever, headache, myalgia
		Toscana	Phlebotomus	Meningitis
		Rift Valley	Mosquito	Encephalitis, hemorrhagic fever
Reoviridae	Coltivirus	Colorado Tick Fever	Tick	Meningitis, exantema
	Seadornavirus	Banna	Mosquito	Encephalitis, flu-like syndrome
Orthomyxoviridae	Thogotovirus	Dori, Thogoto	Tick	Encephalitis
Rhabdoviridae	Vesiculovirus	Indian Vesicular Stomatitis	Phlebotomus	Flu-like syndrome, vesicular stomatitis
		New Jersey Vesicular Stomatitis	Phlebotomus	Flu-like syndrome, vesicular stomatitis

Modified from Moroni et al, Malattie Infettive, 7th edition

Blood-sucking arthropods, usually mosquitoes, serve as vectors for transmission. Some arboviruses are transmitted by ticks or other insect vectors such as sandflies. Because exposure to mosquitoes or ticks is the risk factor for transmission, the number of infections is highest in summer and early fall, consistently with high mosquito populations (Box 1) .
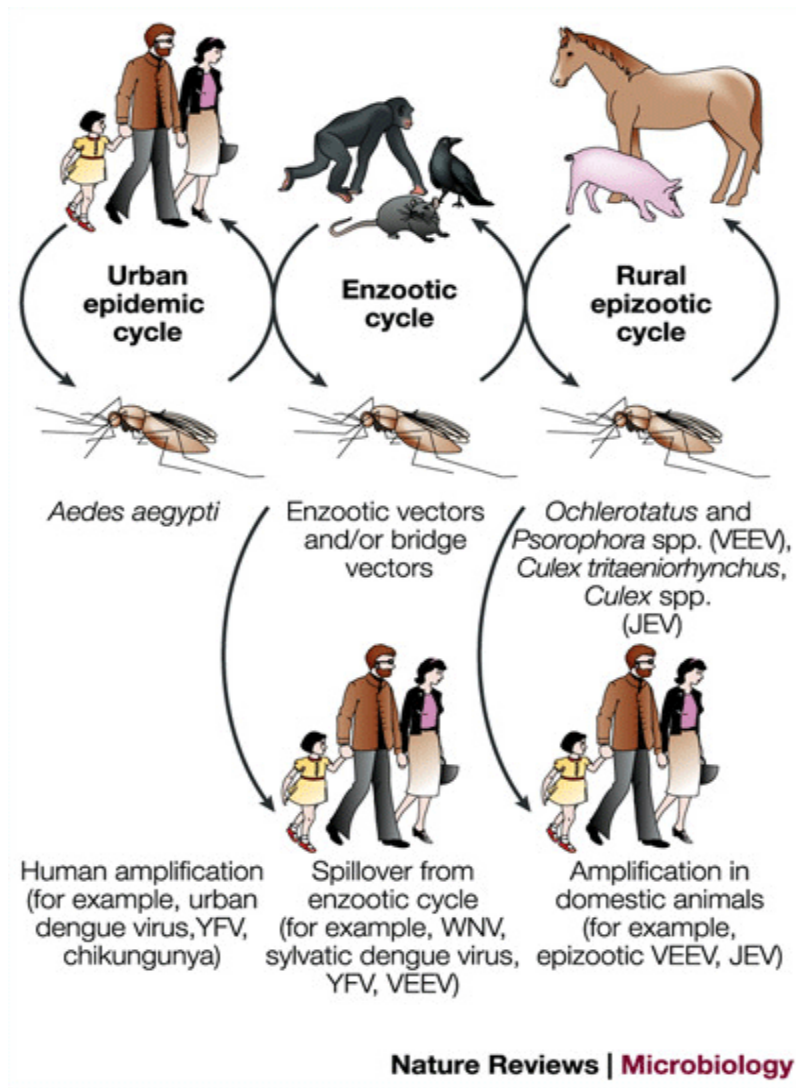
Box 1: *from 'Transmission cycles, host range, evolution and emergence of arboviral disease',Scott C. Weaver & Alan D. T. Barrett,Nature Reviews Microbiology 2, 789-801 (October 2004)*

Some of the important arboviruses include the eastern and western equine encephalitis viruses, from the Togavirus family; St. Louis encephalitis, West Nile, Japanese B, and Murray Valley viruses, from the Flavivirus family; California group and Jamestown Canyon viruses, from the Bunyaviridae family. Colorado tick fever is caused by a coltivirus in the western regions of the United States.

Clinical features of infection include febrile diseases and hemorrhagic fevers. The most common clinical manifestation of the CNS is meningoencephalitis rather than pure meningitis.

Seizures are more common with arboviral meningitis than with any other group of viruses.

Some agents preferentially infect certain age groups, such as St. Louis encephalitis, which affects the elderly, and La Crosse/California serogroup virus, which infects young children. Children with St. Louis or California group encephalitis viruses may not exhibit any neurologic signs or altered mental status.

St. Louis encephalitis (SLE) virus is the most common cause of arboviral meningitis, and is also the most common mosquito-transmitted disease in the United States.

Japanese B encephalitis virus, the most common pathogen in epidemic viral meningitis worldwide, accounts for more than 35,000 infections annually throughout Asia but is estimated to cause 200-300 times that number of subclinical infections.

Of the arboviruses, West Nile virus caused much attention, as it was first recognized in the United States only in 1999 and quickly became an epidemic in 2002, with more than 4,000 reported cases. In 2008, 1,356 cases were reported.[13]

Infection with the West Nile virus is usually asymptomatic or manifests as mild symptoms of nonspecific fever, myalgia, and fatigue. Symptoms are therefore similar to St Louis encephalitis. However, 1 in 150 cases develops a severe disease involving the nervous system, with encephalitis reported more than meningitis. In 2008, 687 cases of West Nile neuroinvasive disease were reported to the US Centers for Disease Control and Prevention (CDC) from all across the United States. [14]

In Europe, there is a history of recognized WNV outbreaks, characterized by human neuroinvasive disease, dating back to the mid-1990s. [15] However, since 2008, there has

been an unprecedented increased WNV activity, including the sustained emergence of a lineage 2 WNV, with a rapid rise in the number of cases of neuroinvasive disease of animals and humans. [16,17] In 2012, there was a peak of 937 WNV cases in Europe and surrounding countries, with ongoing activity in 2013, with preliminary data reporting 783 WNV human cases, including 86 in Greece and 302 in Serbia. [18]

Neuroinvasive West Nile disease occurs more often in elderly persons.

Toscana Virus

Toscana virus (TOSV) is an arthropod-borne virus, identified in 1971, from *Phlebotomus perniciosus* and *Phlebotomus perfiliewi* in central Italy. TOSV belongs to the Phlebovirus genus within the *Bunyaviridae* family. As other bunyaviruses, the genome of TOSV consists of 3 segments (S for small, M for Medium, and L for Large) respectively encoding non structural and capsid proteins, envelope structural proteins, and the viral RNA-dependant RNA-polymerase. It is transmitted by sand flies. Therefore its distribution is bound to that of the arthropod vectors, and virus circulation peaks during summertime when sand fly populations are active. The first evidence of its pathogenicity in humans, specifically its propensity to cause central nervous system (CNS) infections such as meningitis and encephalitis, was reported in central Italy. Only after 2000, it was recognized that TOSV had a much larger geographic distribution than previously believed, and was present in most of the Western European countries located on the northern border of the Mediterranean Sea (Portugal, Spain, France, Greece, Croatia) as well as eastern countries such as Cyprus and Turkey. In the countries where TOSV is present, it is among the three

most prevalent viruses causing meningitis during the warm season, along with enteroviruses and herpesviruses. Up to now, epidemiological data concerning Northern Africa and other countries located south of the Mediterranean are scarce. TOSV must be considered as an emerging pathogen. Despite the important role played by TOSV in CNS infections, it remains a neglected agent and is rarely considered by physicians in diagnostic algorithms of CNS infections and febrile illness during the warm season, probably because of the lack of information. [19]

FIGURE 1

Distribution of (a) Toscana, (b) Sicilian, and (c) Naples viruses in the European Union and neighbouring countries around the Mediterranean Sea up to 2009

Countries with confirmed cases are depicted in mid grey, the estimated distribution limits are depicted with a dark grey line.
Source: V-borne project; reproduced with permission from the European Centre for Disease Prevention and Control.

Adapted from Depaquit J et al, Eurosurveillance, Volume 15, Issue 10, 11 March 2010

Mumps

A member of the *Paramyxoviridae* family, mumps virus was one of the first known causative agents of meningitis and meningoencephalitis.[20]

The incidence of mumps in the vaccination era has decreased significantly to 1 per 100,000 population in the United States.

Nonetheless, outbreaks have occurred in vaccinated populations, including a large epidemic in the United Kingdom that peaked in 2005 and several outbreaks in the American Midwest in 2006.[21] In addition, mumps continues to cause 10-20% of meningitis and meningoencephalitis cases in parts of the world where vaccines are not readily accessible.

Males 16-21 years of age are at highest risk for developing this infection, with a 3:1 male/female ratio.

Clusters of cases occur in schools and colleges in the winter months.

Concomitant parotitis is a helpful clinical tool, but it may be absent in as many as half of cases with CNS involvement.

A cohort study of 12,000 unvaccinated children from northern Finland revealed that mumps meningoencephalitis accounted for 40.9% of all viral CNS infections. Mumps also remains an important cause of aseptic meningitis in England and Japan.

In 2003, epidemics of aseptic meningitis following measles, mumps, rubella (MMR) vaccination campaigns in various nations (including Brazil and the UK) prompted the Global Advisory Committee on Vaccine Safety to conduct a review of vaccine-derived mumps meningitis.[22] At the time, the committee stated that certain strains of the mumps vaccine (Urabe, Leningrad-Zagreb, and Leningrad-3 strains) were associated with higher incidences of post-vaccination aseptic meningitis. In 2006, the committee determined that the international literature reviewed was actually inconclusive and that further studies were needed.[23] Even so, replacement mumps components were developed and vaccines were reformulated worldwide.

Herpes family viruses

Herpes simplex virus HSV-1, HSV-2, varicella-zoster virus (VZV), Ebstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus-6 (HHV-6) collectively cause approximately 4% of cases of viral meningitis, with HSV-2 being the most common offender. The viruses may attack at any time of the year.

Meningitis caused by these viruses is often self-limited. When associated with encephalitis, however, the mortality rate can be high. Early treatment with acyclovir can significantly reduce morbidity.

HSV-1 remains the most common cause of sporadic encephalitis, while HSV-2 infections of CNS are mostly restricted to aseptic meningitis.

HSV-2 genital infection may precede meningitis; sexual contact with actively infected individuals is one of the known risk factors.

In one review, however, only 3 of 23 patients with HSV-2 meningitis had a history of prior genital herpes or had genital lesions noted at the time of presentation.[24] Maternal-fetal transmission of HSV-2 can occur, leading to significant systemic sequelae, including infantile septicemia and death.

EBV, HSV-1, and especially HSV-2 have been associated with Mollaret meningitis, a rare, benign, recurrent type of meningitis that resolves spontaneously. Mollaret cells (activated monocytes with an atypical appearance of enlarged, bilobed nuclei and amorphous cytoplasm) are found in the CSF usually on the first day of symptoms. HHV-6, EBV, and the human immunodeficiency virus (HIV) have also been implicated. These viruses are all known to remain latent within the nervous system.

CMV infections occur mostly in immunocompromised hosts. CMV may cause subacute encephalitis in patients with AIDS. Congenital CMV, which is a much more serious form of infection, has significant associated morbidity and mortality.

Childhood or adult chickenpox infections by VZV rarely are complicated by meningitis. Adult zoster involving any dermatome may lead to meningitis or meningoencephalitis.

Lymphocytic choriomeningitis virus

LCMV belongs to arenavirus family which is characterized by a single-stranded RNA genome. Now a rare cause of meningitis, the virus is transmitted to humans by contact with rodents (eg, hamster, rats, mice) or their excreta, especially urine. Human cases of LCM are most common in autumn. Persons at highest risk of infection are laboratory workers, pet owners, or persons under unhygienic living conditions.

Adenovirus

Adenovirus infections are ubiquitous but these result mostly in subclinical disease. Adenovirus infection is a rare cause of meningitis in immunocompetent individuals but a major one in patients with acquired immunodeficiency syndrome (AIDS). The infection may occur simultaneously with an upper respiratory infection.

Measles

This Morbillivirus is another causative agent of meningitis which has become rare. Only 1 in 1000 to 2000 patients with measles develops clinical signs of encephalitis. The typical

maculopapular rash on patients with measles is very useful in the clinical diagnosis. Most cases occur in younger people in schools and colleges. Still a worldwide health threat, measles has the highest attack rate of any infection. Eradication of measles is an important goal of the World Health Organization (WHO).

HIV

Meningitis is multifactorial in patients with HIV or AIDS. Aseptic meningitis may be caused by HIV itself and in this case it has atypical features such as chronicity and recurrence. About the time of seroconversion, patients may present with CSF pleocytosis, elevated protein level and, occasionally, high intracranial pressure.

Reports have suggested that as many as 5-10% of HIV infections can be heralded by meningitis. Aside from the usual meningeal signs, HIV infections may also cause global encephalopathy, seizures, and focal neurologic deficits. Some patients develop chronically abnormal CSF findings with mild or no symptoms. HIV often can be isolated from the CSF.

Others

Other causative agents of meningitis include Rabies virus which usually causes encephalitis but in some cases may cause meningitis too, Rotavirus, Influenza and Parainfluenza virus. However these are very rare and often fatal.

Pathophysiology and clinical manifestations

CNS invasion may occur via several mechanisms but most viral pathogens gain access via either of two main routes: hematogenous and neural. Most neurotropic viruses first multiply at extraneural sites (initially at the portal of entry), establish viremia, and then cross the blood-brain barrier (BBB) to invade the CNS. [25, 26] Neural penetration refers to spread along nerve roots and is usually limited to herpes viruses (HSV-1, HSV-2, VZV) and possibly some enteroviruses.

Multiple host barriers prevent viral inoculum from causing clinically significant infection. These include local and systemic immune responses, skin and mucosal barriers, and the blood-brain barrier (BBB).

The virus replicates in the portal of entry (ie, respiratory or gastrointestinal mucosa) and gains access to the bloodstream. Primary viremia introduces the virus to the reticuloendothelial organs (liver, spleen, lymph nodes). If the replication persists despite immunologic defenses, secondary viremia occurs, which is thought to be responsible for seeding of the CNS. Rapid viral replication likely plays a major role in overcoming host defenses.

The actual mechanism of viral penetration into the CNS is not fully understood. The virus may cross the BBB directly at the capillary endothelial level or through natural defects, such as the area postrema and other sites that lack a BBB.

The inflammatory response is seen in the form of pleocytosis; polymorphonuclear leukocytes (PMNs) lead the differential cell count in the first 24-48 hours, followed later by increasing numbers of monocytes and lymphocytes. The CSF lymphocytes have been

recognized as T cells, although B cell immunity is also important in defending against some viruses.

Evidence exists that some viruses gain access to the CNS by retrograde transport along nerve roots. For example, the likely pathway for HSV-1 encephalitis is via the olfactory or trigeminal nerve roots, with the virus being transported by the olfactory fibers to the basal frontal and anterior temporal lobes.

Upon presentation, most patients report fever, headache, irritability, nausea, vomiting, nuchal rigidity, rash, or fatigue within the previous 18-36 hours. Constitutional symptoms of vomiting, diarrhea, cough, and myalgias appear in more than 50% of patients.

For several weeks or longer, children may experience irritability, incoordination, and an inability to concentrate.

Headache is almost always present in patients with viral meningitis and is often reported as severe. However, the classic description of abrupt onset of the "worst headache of my life," attributable to aneurysmal subarachnoid hemorrhage, is uncommon.

History of temperature elevation occurs in 76-100% of patients who come to medical attention. A common pattern is low-grade fever in the prodromal stage and higher temperature elevations at the onset of neurological signs.

Younger children may not report headache and may simply be irritable.

Newborns may present with poor feeding and lethargy.

Some viruses cause rapid onset of the above symptoms, while others manifest as nonspecific viral prodromes, such as malaise, myalgia, and upper respiratory symptoms. In many cases, symptoms have a biphasic pattern; the nonspecific flu-like symptoms and low-grade fever precede neurologic symptoms by approximately 48 hours. With the onset of neck stiffness and headache, the fever usually returns.

Meticulous history taking is essential and must include evaluation of exposure to ill contacts, mosquitoes, ticks, outdoor activity in areas of endemic Lyme disease, travel history with possible exposure to tuberculosis, as well as history of medication use, intravenous drug use, and sexually transmitted disease risk.

Some general physical findings in viral meningitis are common to all causative agents.

The classically taught triad of meningitis consists of fever, nuchal rigidity, and altered mental status, but not all patients have all 3 symptoms.

Fever is very common (80-100% of cases) and usually ranges from 38°-40°C.

Neck stiffness or other signs of meningeal irritation (Brudzinski or Kernig sign) may be seen in more than half of patients, but these symptoms are generally less severe than they are in bacterial meningitis. Pediatric patients, especially neonates, tend not to exhibit nuchal rigidity on examination.

Irritability, disorientation, and altered mentation may be seen.

Severe lethargy or bulging fontanelle in neonates is a sign of increased intracranial pressure but may be absent in more than half of all cases. The neonate may exhibit hypotonia, irritability, and poor feeding. The clinical picture can mimic neonatal bacterial septicemia accompanied by multiple organ system involvement.

Headache is common and is characteristically severe.

Photophobia is relatively common but may be mild. Phonophobia may also be present.

Seizures can occasionally occur and are usually the result of the fever, although the involvement of brain parenchyma (encephalitis) should be considered.

Global encephalopathy and focal neurologic deficits are rare but can be present. Deep tendon reflexes are usually normal but may be hyperactive.

Various signs of specific viral infection can aid in diagnosis. These include the following:

- Pharyngitis and pleurodynia in enteroviral infections

- Skin manifestations, such as zoster eruption from VZV, maculopapular rash from measles and enteroviruses, vesicular eruption from herpes simplex, and herpangina from coxsackievirus A infections

- Pharyngitis, lymphadenopathy, and splenomegaly, which suggest EBV infection

- Immunodeficiency and pneumonia, which should suggest adenovirus, CMV, or HIV as the causative agent

- Parotitis and orchitis, from mumps

- Gastroenteritis and rash, which occur with most enteroviral infections

The prognosis for viral meningitis is usually excellent, with most cases resolving in 7-10 days. Implicit in the diagnosis is the self-limited nature of this disease. The exception falls with the neonatal patients, in whom viral meningitis can be fatal or associated with significant morbidity.

Concomitant encephalitis adds significant potential for adverse outcomes. Concurrent systemic manifestations, such as pericarditis and hepatitis, are other indicators of poor prognosis.

Diagnosis

General assessment

Routine chemistry and hematology tests, arterial blood gas analysis, coagulation studies, and liver function tests should be performed.

The serum white blood cell (WBC) count is not a sensitive indicator of the severity of infection, especially in the immunocompromised, neonatal, or elderly patient. However, a high WBC, particularly neutrophils, suggests a bacterial etiology.

The serum sodium level may be abnormal because of dehydration or the rare occurrence of syndrome of inappropriate antidiuretic hormone secretion (SIADH). Since abnormal sodium levels may contribute to neurological symptoms, these must be followed up and corrected if needed.

The serum amylase level may be elevated in cases of viral meningitis that are caused by mumps, even in the absence of parotitis.

Reports have shown high C-reactive protein (CRP) levels in the serum of children with bacterial meningitis whose CSF Gram stain findings were negative for bacteria. However, a comparable group of children with viral meningitis did not have similar elevations in serum CRP (i.e., 50-150 in bacterial meningitis group vs < 20 in the viral meningitis group).

Microscopy of CSF should be performed to better define the nature of white cells and detect bacteria and fungi.

All patients whose condition is not improving clinically within 24-48 hours should have a more extensive workup to discern the cause of meningitis.

Cerebrospinal fluid examination

CSF examination is the most important test in establishing the cause of meningitis. Prior to lumbar puncture (LP), a computed tomography (CT) scan should be performed in patients with any abnormal neurologic sign, to rule out an intracranial lesion or obstructive hydrocephalus.

A high WBC count in the CSF (especially neutrophils), a high protein level, and a low glucose level should suggest a diagnosis of bacterial meningitis, although some viral pathogens may produce similar CSF profiles. In fact, pleocytosis with WBC counts in the range of 50 to more than 1,000 x 10^9/L of blood has been reported in viral meningitis. Mononuclear cell predominance is the rule, but PMNs may make up most cells in the first 12-24 hours; the cell count is usually then dominated by lymphocytes in the classic CSF pattern of viral meningitis. This helps to distinguish viral from bacterial meningitis, which has a much higher cell count and a predominance of PMNs in the cell differential, although this is not an absolute rule. In fact, a retrospective multicenter study found that neither the presence nor quantity of immature neutrophils (bands) in CSF independently predicted bacterial meningitis among children with CSF pleocytosis.[27]

The CSF protein level usually is only slightly elevated, but it can range from normal to as high as 200 mg/dL.

The glucose level is normal in most cases, but severe hypoglycorrhachia has been reported, especially with LCMV or the mumps virus. Very low glucose levels with a lymphocytic pleocytosis may be seen in tuberculous meningitis.

Etiologic diagnosis

Blood, feces, and throat swabs need be sent for viral serology and cultures.

Serum titers of antibodies against possible etiologic infective agents should be obtained at admission. Additional serum collection 10-21 days later may aid in discerning rising titers in the antibodies against specific viral pathogens; a 4-fold increase in viral antibodies confirms the diagnosis. This is particularly useful for arboviral and LCMV cases, but it also is helpful in ruling out toxoplasmosis, leptospirosis, borreliosis, and rickettsial infections. Although some of these studies do not yield an immediate result for clinical decision making, they may be useful for prognostication.

Real-time PCR testing for enterovirus was first cleared for marketing in 2007 by the US Food and Drug Administration (FDA) and is now available through commercial laboratories.[28] Results are available in approximately 3 hours, as opposed to days to weeks in traditional PCR studies. In clearing the test, the FDA cited a multicenter study in which 96% of patients who tested positive did have viral meningitis, and 97% of patients who tested negative did not have viral meningitis. In a retrospective study of routine PCR testing of CSF for enterovirus—using a test with turnaround time of 23 hours— confirmation of enteroviral meningitis by PCR decreased the length of hospitalization and the duration of antibiotic use among infants aged 90 days or younger.[29] Among 22 children with meningitis due Enterovirus, in 10 patients, genome was detected in CSF only; in 3 patients in serum only, and in 9 patients in both. Therefore, the test on CSF seems to be much more sensitive, but the test on serum might detect an additional small group of cases. [30]

In recent years, PCR on CSF has become the gold standard for the diagnosis of viral infections of the CNS. Rapid viral clearance was typical for HSV, VZV, CMV, and EV infections, although the maximum duration of viral detection was 15days for HSV and 12days for VZV, respectively. This suggests that the detection of HSV, VZV, CMV, and EV strongly indicates symptomatic viral CNS disease. [31]

If the results of PCR testing of the CSF and the viral culture for herpes simplex are negative, acyclovir can be discontinued; otherwise, a 10-day course is recommended.

Imaging

Viral meningitis is usually not associated with imaging abnormalities, thus imaging tests are not helpful in establishing a diagnosis or etiology. CT scanning is performed to exclude contraindications for lumbar puncture, such as increased intracranial pressure, but whether it should be performed in all patients or in selected cases remains controversial.

In patients in whom encephalitis is suspected, MRI with contrast enhancement and adequate visualization of the frontal and temporal areas, typically affected by HSV, is necessary.

Electroencephalography (EEG) may be performed if encephalitis or subclinical seizures are suspected in the altered patient. Periodic lateralized epileptiform discharges (PLEDs) are often seen in herpetic encephalitis.

Differential Diagnosis

Since viral meningitis presents as aseptic meningitis upon CSF examination a differential diagnosis with other etiological factors should be taken into consideration. Such factors include infectious agents like *Treponema pallidum*, *Borrelia burgdorferi* and *Mycobacterium tuberculosis*, autoimmune diseases, medications or even partial antibiotic treatment for bacterial meningitis. However these account for only a small proportion of cases so careful history taking and laboratory findings should help to rule them out.

Treatment

Treatment for viral meningitis is mostly supportive. Rest, hydration, antipyretics, and pain or anti-inflammatory medications may be given as needed. The most important decision is whether to initiate antimicrobial therapy empirically for bacterial meningitis while waiting for the cause to be identified. Intravenous (IV) antibiotics should be administered promptly if bacterial meningitis is suspected.

No surgical therapy is usually indicated in patients with viral meningitis. In rare patients in whom viral meningitis is complicated by hydrocephalus, a CSF diversion procedure, such as ventriculoperitoneal (VP) or LP shunting, may be required. Ventriculostomy with an external collection system is indicated in the rare cases of acute hydrocephalus.

Patients with signs and symptoms of meningoencephalitis should receive acyclovir early to possibly curtail HSV encephalitis. Therapy can be modified as the results of Gram stain, cultures, and PCR testing become available. Patients in unstable condition need critical care unit admission for airway protection, neurologic checks, and the prevention of secondary complications.

Enteroviruses and HSV are both capable of causing viral septic shock in newborns and infants. In these young patients, broad-spectrum antibacterial coverage and acyclovir should be instituted as soon as the diagnosis is suspected. Special attention should be paid to fluid and electrolyte balance (especially sodium), since SIADH has been reported. Fluid restriction, diuretics, and, rarely, hypertonic saline infusion may be used to correct the hyponatremia. Prevention of secondary infections of urinary tract and pulmonary systems is of paramount importance.

Waiting for LP results should not delay administration of antibiotics when warranted on clinical grounds. Broad-spectrum coverage is attained with ampicillin and a third-generation cephalosporin (ceftriaxone or cefotaxime; ceftazidime can also be used). Aminoglycosides are used in severe infections in neonates or children. Antituberculous, antifungal, and antiretroviral medications are reserved for clinically suggested or laboratory-confirmed cases.

Seizures should be treated immediately with IV anticonvulsants, such as lorazepam, phenytoin, midazolam, or a barbiturate. Unconscious patients with viral encephalitis may be in non-convulsive status epilepticus, and EEG is used to reveal and monitor subclinical seizures.

Cerebral edema does occur in cases of severe encephalitis and may require intracranial pressure control by infusion of mannitol, IV dexamethasone, or intubation and mild

hyperventilation, with arterial PCO2 around 28-30 mm Hg. Placement of an intracranial pressure monitor with transduced intraparenchymal pressure is recommended in these cases. Multiple antiviral medications are currently being tested in the general population; their impact on preventing the sequelae of viral meningitis and encephalitis has not yet been established. In herpetic viral infections, acyclovir is significantly beneficial only if given very early in the course of the infection. Suspected cases should be treated as soon as possible; in cases complicated by seizures, encephalitis is assumed and acyclovir should be initiated.

Ganciclovir is used for severe CMV-related infections such as congenital infection or infection in immunocompromised hosts, including hematologic patients and transplant recipients.

Administration of IV Ig to neonates with overwhelming enteroviral meningitis has met with occasional success and is reserved for severe cases lacking other therapeutic options.

Prognosis

Viral meningitis is typically a benign disease which resolves spontaneously or with appropriate treatment in 7-10 days. In small children and immunocompromised patients though it can more often bring complications such as seizures, blindness, hearing loss, paralysis or hydrochephalus.

Objectives

The main aim of this study was to gather epidemiological data related to viral infections of the CNS as well as laboratory findings which could suggest the etiological agent responsible for the disease.

Secondarily, we also aimed at characterizing viral strains, particularly those of emerging diseases, such as dengue fever virus, West Nile virus and Toscana virus, correlating them with epidemiology, clinical and immunological features.

Methods

Patients referred to Emergency Department and the Infectious Diseases Unit of the Azienda Ospedaliera Universitaria Pisana were screened. Patients who had signs of CNS infection underwent a lumbar puncture. Time of LP after onset of symptoms was established.

Patients were classified as having meningitis if they had neck stiffness, other signs of meningeal irritation or laboratory evidence of inflammation at CSF examination, encephalitis if they had focal signs, impaired consciousness or seizures, meningoencephalitis if they had both.

A check list of serologic and molecular virology and microbiology tests to be performed on blood and cerebrospinal fluid of patients with aseptic infection of the CNS was developed and distributed to the Infectious Diseases clinicians (Figure 2).

Figure 2. Check List for the etiologic diagnosis of aseptic infection of the Central Nervous System infections

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TESTS ON SERUM

Antibodies

☐ Widal-Wright

☐ *Listeria monocytogenes*

☐ *Borrelia burgdorferi*

☐ *Leptospira spp*

☐ *Mycoplasma pneumonia*

☐ *Chlamydia pneumonia*

☐ *Coxiella burnetii*

☐ *Bartonella henselae*

☐ *Rickettsia conori*

☐ *Rickettsia typhi*

☐ lue screening

☐ Toscana virus

☐ HSV 1 and 2, VZV, CMV, EBV, HHV 6, HHV 7

☐ Coxsackie virus, Echovirus,

☐ Adenovirus

☐ HIV

☐ West Nile

☐ Tick Born Encephalitis (TBE)

☐ Lymphocitic Choriomeningitis Virus (LCMV)

□ Parotitis

□ Rubella

□ Parvovirus B19

□ HTLV I/II

□Chikungunya

□ **Quantiferon**

□ **Cryptococcus antigen**

TESTS ON CSF

□ **Physical-chemical analysis**

□ **Culture and methilene blue stain**

Genomes

□ HSV 1 and 2, VZV, CMV, EBV, HHV 6, HHV 7

□ Enterovirus

□ Adenovirus

□ Human Respiratory Syncytial Virus

□ HIV

□ West Nile

□ Chikungunya

□ Parvovirus B19

Antibodies

□ *Borrelia burgdorferi*

□ Toscana virus

☐ HSV 1 and 2, VZV, CMV, EBV, HHV 6, HHV 7

☐ Coxsackie virus, Echovirus

☐ Adenovirus

☐ HIV

☐ West Nile

☐ TBE

☐ LCMV

☐ Parotitis

☐ Rubella

☐ Parvovirus B19

☐ HTLV I/II

☐ Chikungunya

☐ **PCR, culture and ZN stain for mycobacteria**

☐ **Cryptococcus antigen**

OPTIONAL

☐ Influenza genome on pharingeal and nasal swab during epidemic season

☐ Complete syphilis serology on serum and CSF in case of positive screening or HIV positive patient

☐ HSV 1 and 2 or VZV genome on vesicular cutaneous lesions

☐ JC genome in CSF of immunocompromised patients

☐ Toxoplasma serology on serum and genome in CSF of immunocompromised patients with focal lesions

□ Serology of Dengue and other Arbovirus based on epidemiology

□ Genome, culture and ZN stain on sample other than CSF

□ Incomplete antibodies for Brucella

Samples have been processed at the laboratory of the Division of Virology of the Azienda Ospedaliera Universitaria Pisana. Tests were performed following a priority algorithm.

Comprehensive biochemical and immunological tests, including lymphocyte subpopulation analysis, protein electrophoresis and immunoglobulin subclasses determination, were performed.

Neuroimaging procedures were conducted according to clinical indications.

Epidemiological, laboratory and clinical data were collected using a specifically designed, dedicated database.

Patients were followed up for at least 6 months.

Samples of blood and cerebrospinal fluid were stored at -80°C.

Data from the period 2009 to 2015 was analyzed.

Additionally, a seroprevalence survey for Toscana Virus was conducted on consecutive patients admitted in the Infectious Diseases Unit at admission, regardless the symptoms.

Results

Overall, in the period 2009 to 2015, 68 cases have been diagnosed. Males were 40/68 (58.8%), mean age was 49.6 (range 18-85). Age distribution is reported in figure 3.

Figure 3. Age distribution of patients with viral infection of the Central Nervous System, Pisa, 2009-2015
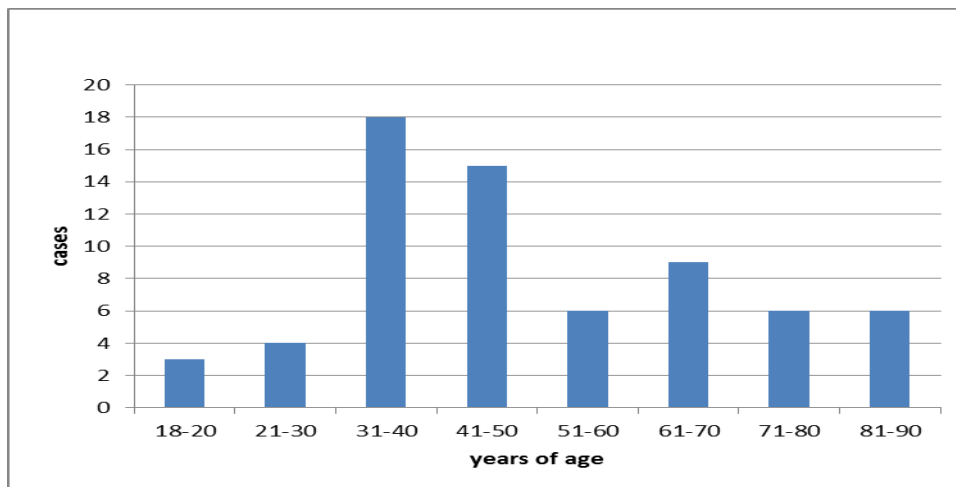
Etiology was established in 34/68 (50.0%) as follows: VZV 9 cases, Toscana Virus 7, HSV-1 6, HSV-2 and Enterovirus 5, West Nile 2, HHV 7 and parvovirus B19 one (table 1).

When lumbar puncture was performed within 72h from the onset of symptoms, an etiologic diagnosis was made in 17/30 cases (56.6%); when lumbar puncture was performed beyond 72h from the onset of symptoms, an etiologic diagnosis was made in 11/32 cases (34.3%).

LP	Diagnosed cases	Undiagnosed cases	Total	%
Within 72h	17	13	30	56.6%
After 72h	11	21	32	34.3%
Total	28	34	62	45.1%

In case of VZV, PCR on CSF was positive in 6/9 cases; in 6 cases, including those with negative PCR on CSF, CNS involvement occurred in association with varicella or zoster and PCR from vesicles was positive in all cases. In case of HSV-1, PCR on CSF was positive in 5/6 cases; in that with negative result, PCR was positive on serum. In case of HSV-2, PCR on CSF was positive in 2/3 cases; in that with negative result, PCR was positive on serum. In 7/7 cases of Toscana virus infection, diagnosis was based on detection of IgM on serum; IgM on CSF were also detected in 3/7 cases; in one case PCR on CSF was also performed and turned negative. In case of Enterovirus, PCR on CSF was positive in three cases and neutralizing antibodies at high titer positive in the remaining two cases. In case of West Nile virus, diagnosis was based on detection of IgM on serum. In case of HHV-7, PCR was positive on CSF. In case of parvovirus B19, PCR was positive on CSF.

Table 1. Etiology of viral infections of the Central Nervous System, Pisa, 2009-2015

Etiology	N	%
Varicella Zoster Virus	9/68	13.2
Toscana Virus	7/68	10.2
Herpes simplex Virus 1	6/68	8.8
Enterovirus	5/68	7.3
Herpes Simplex Virus 2	3/68	4.4
West Nile Virus	2/68	2.9
Parvovirus B19	1/68	1.4
Human Herpes Virus 7	1/68	1.4
Unknown	35/68	51.4

Figure 4: Etiology of viral infections of the Central Nervous System, Pisa 2009-2015
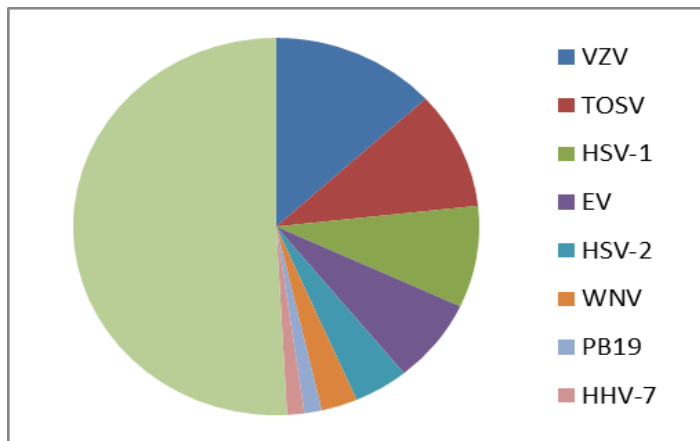
Clinical classification was as follows: 37 patients had meningitis, 26 had meningoencephalitis, 2 had myelitis, one had encephalitis, one had meningoencephalomyelitis and one had meningoradiculoneuritis (table 2). Among patients with meningitis, 5 had a Toscana virus infection, 3 had a HSV-2 infection, including one case of relapsing HSV-2 meningitis, namely Mollaret syndrome, 4 had Enterovirus, 1 HHV-7, 1 West Nile virus, 1 parvovirus B19, 23 an infection of unknown etiology. Among patients with meningoencephalitis or meningoencephalomyelitis, 8 had VZV infection, 3 HSV-1, 2 Toscana virus, 1 Enterovirus, 11 an infection of unknown etiology. One patient with meningoradiculoneuritis had VZV infection and one patient with myelitis West Nile Infection.

Table 2. Clinical classification of viral infections of the Central Nervous System, Pisa, 2009-2015

Clinical classification	N	%
Meningitis	37/68	54.4
Meningoencephalitis	26/68	38.2
Myelitis	2/68	2.9
Meningoencephalomyelitis	1/68	1.4
Meningoradiculoneuritis	1/68	1.4
Encephalitis	1/68	1.4

Mean length of stay was 12.1 days.

All patients lived in the catchment area of the Hospital, apart one tourist from Belgium.

Impaired immunity was detected in 17 cases: diabetes in 5 cases, malignancy, renal failure immunosuppressive therapy and HIV in 2 cases, splenectomy, alcoholism, hypogammaglobulinemia, idiopathic CD4 lymphocyte reduction in one case (table 3). Among the 16 patients with some kind of immunosuppression, 6 cases had VZV infection, 3 HSV-1 infection, one Toscana virus infection and 6 an infection of unknown etiology.

Table 3. Demography and comorbidities of patients with viral infections of the Central Nervous System, Pisa, 2009-2015

	N	%
Males	40/68	58.8
Females	20/68	41.2
Mean age	49.6 (18-85)	
Immunosuppresive condition		
Diabetes	5/68	7.3
Immunosuppressive treatment	2/68	2.9
Malignancy	2/68	2.9
HIV	2/68	2.9
Hypogammaglobulinemia	1/68	1.4
Alcohol abuse	1/68	1.4
Renal failure	2/68	2.9
Asplenism	1/68	1.4
Idiopathic CD4 deficit	1/68	1.4

Other comorbidities		
Migraine/recurrent headache	9/68	13.2
Chronic Cerebral Ischemia	4/68	5.8
Hypertension	10/68	14.7
Body weight excess/Obesity	4/68	5.8
Rheumatoid arthritis	2/68	2.9
Atopic dermatitis	1/68	1.4
Psoriasis	1/68	1.4

At least two among fever, headache, impaired consciousness, focal signs and neck stiffness were present at admission in all patients but two, one with West Nile myelitis and one with VZV meningoencephalitis and zoster. In particular, fever occurred in 60/68 (88.2%), headache in 48/68 (70.5%), neck stiffness in 37/68 (54.4%), impaired consciousness in 25/68 (36.7%), seizures in 9/68 (13.2%), focal signs in 5/68 (7.3%) (table 4).

Table 4. Clinical features of viral infections of the Central Nervous System, Pisa, 2009-2015

Clinical manifestation	N	%
Fever	60/68	88.2
Headache	48/68	70.5
Neck stiffness	37/68	54.4
Impaired consciousness	25/68	36.7
Seizures	9/68	13.2
Focal signs	5/68	7.3

Plain CT scan was negative in all cases but one case of HSV-1 encephalitis who showed a hypodensity in the temporal lobe and finally died. Magnetic resonance showed findings consistent with infection in 12/30 (40.0%); signs of cerebral parenchymal involvement were found in 6/27 (22.2%) cases of meningoencephalitis, 5 due to HSV-1 and one due to VZV. EEG revealed abnormalities in 39/56 (69.6%) (table 5). Localized abnormalities were found in 21/28 (75%) cases of meningoencephalitis or meningoencephalomyelitis.

Table 5. Sensitivity of imaging and EEG in the diagnosis of viral infections of the Central Nervous System, Pisa, 2009-2015

Method	Abnormalities	
	N	%
Plain CT scan	1/68	1.4
Magnetic Resonance	12/30	40.0
EEG	39/56	69.6

At admission, C-reactive protein was altered (>0.5 mg/dl) in 42/64 (65.6%); procalcitonin was slightly above normality (>0.5 mg/dl) in 2/38 (5.2%) cases: one VZV encephalitis and one Toscana virus meningitis. WBC was increased (>11,000x10^9/L) in 25/68 (36.7%); an increase in the neutrophil count (>7,500x10^9/L) was observed in 24/68 (35.2%), an increase in the lymphocyte count (>3,500x10^9/L) was observed in 1/68 (1.4%).

In 9/61 (14.7%) patients a reduced absolute count of CD4 lymphocytes (<410x10^9/L) was detected; in 9/61 (14.7%) patients a reduced absolute count of CD8 lymphocytes

($<190\times10^9$/L); in 9/61 (14.7%) patients a reduced absolute count of NK lymphocytes ($<90\times10^9$/L).

Only 5/51 (9.8%) patients had a reduced level of IgG (<700 mg/dl), none had a reduced level of IgA or IgM.

C3c was slightly below normal (90 mg/dl) in 2/17 (11.7%) cases; C4 was slightly below normal (10 mg/dl) in 2/17 (11.7%) cases as well (table 6).

Table 6. Laboratory features of viral infections of the Central Nervous System, Pisa, 2009-2015

Parameter	N	%
C reactive protein increase	42/64	65.6
Procalcitonin increase	2/38	5.2
WBC increase	25/68	36.7
Neutrophils increase	24/68	35.2
CD4+ lymphocyte decrease	9/56	14.7
CD8+ lymphocyte decrease	9/56	14.7
NK lymphocyte decrease	9/56	14.7
IgG decrease	5/51	9.8
C3c decrease	2/16	12.5
C4 decrease	2/16	12.5

At CSF examination, leukocytes count was increased ($>5 \times 10^9$/L) in 65/67 (97%) and lymphocytes were prevalent in 63/67 (94.0%); glucose was reduced (<50 mg/dl) in 12/67 (17.9%), but CSF/blood glucose ratio was less than 40% only in 2/67 (2.9%); proteins were increased (>60 mg/dl) in 45/67 (67.1%) (table 7). Overall mean CSF leukocytes count was 285×10^9/L while mean leukocytes count of Enterovirus infections was 152×10^9/L and that of VZV infections was 625×10^9/L. Mean protein concentration was 106.2 mg/dl while that of VZV infections was 237.9 mg/dl.

Table 7. Cerebrospinal fluid features of viral infections of the Central Nervous System, Pisa, 2009-2015

Feature	N	%
WBC count increase	65/67	97.0
Glucose decrease	12/67	17.9
CSF/Blood glucose ratio decrease	2/67	2.9
Proteins increase	45/67	67.1

Acyclovir was administered empirically in all cases and discontinued in case HSV genome on CSF was negative. Steroids were administered in 4 cases. Outcome was favorable in all but 5 cases: two died and three had permanent consequences, namely paraparesis in one case and impaired mental status in two cases.

As far as the seroprevalence for Toscana virus is concerned, IgG or IgM were positive in 34/100 cases (34%), with a clear trend to increase with age: 0% from 16 to 20, 22.1% from 21 to 30, 26.7% from 31 to 40, 31.6% from 41 to 50, 25.3% from 51 to 60, 33.3% from 61 to 70, 52.6% above 70. Livorno and Massa Carrara areas resulted to have the highest prevalence (80 and 75% respectively).

Figure 5. Toscana virus seroprevalence by age, AOUP, 2012-13
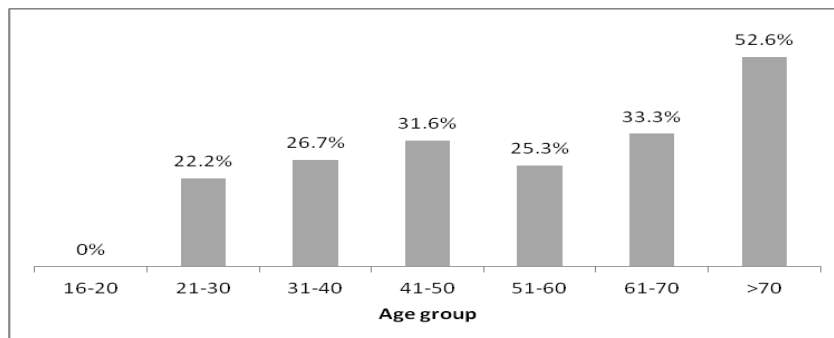
Discussion

Certainly, sample size cannot allow any definitive conclusions. However, in the study period an etiologic diagnosis was made in 50.0% of cases, which is consistent with what others reported. [32, 33]

This result is encouraging and prompts us to provide further efforts in this direction. Revision of existing algorithms, dissemination meetings, preparation of graphic materials such as posters and flow charts, might be considered in the future.

The fact that an earlier lumbar puncture (<72h) was associated to a higher probability of detecting viral genome in CSF (56.6% versus 34.3%) although statistically not significant (p=0.0812) should prompt us not to delay such a crucial procedure. A higher number of cases may be necessary to achieve a significant value.

Clinical relevance of viral infections of the CNS justifies an aggressive diagnostic approach. It has been reported that the realization of a brain scanner before the lumbar puncture was associated with a significant delay in the realization of the lumbar puncture in the Emergency Unit. [33] This is consistent also with our case series, because almost all patients had undergone plain CT scan before lumbar puncture, also when no strict indications existed. However, among patients who underwent lumbar puncture beyond 72 hours from the onset of symptoms, the delay was mainly due to delay in accessing the hospital, that means general practitioners should also be sensitized on the issue.

Overall, the data of the period 2009 to 2015 gives a picture of the local epidemiology of the viral infections of the CNS.

Our study does not allow to obtain data on incidence. However, also considering that our hospital is a referral center, viral infections of the CNS seem to have a limited impact in our communities. Still, we can assume that cases accessing health services are only the most severe ones and many more occur with no or mild clinical manifestations.

Age of patients ranged from 18 to 85 years but almost half of patients had from 30 to 50 years. Notably, mean age of patients having meningitis was 49.6 while mean age of those having involvement of brain parenchyma was 63.6, meaning that older patients more often develop severe forms.

Etiology of our case series generally reflects that reported in the literature for adults, with some peculiarities.

VZV turned out to be the first cause of viral infection of the CNS. Among the 9 patients with VZV infection, all but one had signs of encephalitis. Other case series report Enterovirus to be largely the first cause of meningitis while HSV-1 and HSV-2 to be the first cause of encephalitis, followed by VZV. [34, 31] In 7/9 cases some kind of immunosuppression was revealed. One case of encephalitis reported persistent neurocognitive impairment and one case of meningoradiculoneuritis reported paraparesis. One case occurred during varicella and 5 in association with zoster. This should remind us about the importance of suspecting CNS involvement in such cases, especially in case of immunosuppressed or otherwise fragile patients, to refer the case to hospital and/or timely start antiviral treatment. The live attenuated vaccine for VZV is very effective in preventing varicella and therefore complications of acute disease, including those affecting the CNS. In fact, although cases of meningitis as adverse events following vaccination have been described, incidence is low. [35] However, impact of vaccination on reactivation and its most severe manifestations has not been established. For instance, among 84 children aged

1 month to 18 years, admitted with neurological complications of acute disease, including encephalitis, only 4 had been vaccinated. [36] Instead, out of 7 children with meningitis or encephalitis, 4 had received at least one dose of vaccine. [37] Other cases of CNS infection due to reactivation of vaccine strain have been reported. [38, 39, 40] A live attenuated anti-zoster vaccine has been recently proposed and it has been reported to reduce reactivation of VZV, expressing as zoster, by 50%. [41] Possible effects of this vaccine on VZV neurological manifestations has not been established. However, cases of VZV meningitis following vaccination in elderly have been reported. [42]

HSV-1 and HSV-2 were responsible of 6 and 2 cases respectively. All patients with HSV-1 infection had meningoencephalitis, all cases with HSV-2 infection had meningitis, including one case of Mollaret syndrome. Out of six cases of HSV-1 infection, two occurred in patients with known causes of immunosuppression, namely hypogammaglobulinemia in one case and steroids plus methotrexate for rheumatoid arthritis in the other case; additionally, two other patients were 84 and 85 years old. This is consistent with the existing literature indicating HSV-1 as a typical opportunistic infection. Differently, all three patients with HSV-2 infection had no history or findings suggestive of immunosupression. Two out of six patients with HSV-1 infection died while all the three patients with HSV-2 infection fully recovered. These data confirm HSV-1 and HSV-2 to cause different kinds of involvement of the CNS, with different clinical manifestations and outcome. Treatment of HSV-1 and HSV-2 traditionally relies on acyclovir, since its introduction more than 30 years ago. Resistant strains have been described, but susceptibility tests are not routinely performed, since they are not standardized, time-demanding and their clinical relevance not established. [43] Acyclovir has also been variously prescribed for primary or secondary prophylaxis, but emergence of resistance has been described. [44]

Toscana virus turned out to be the second cause of viral infection of the CNS in our case series, together with HSV-1. All cases occurred during summer season, consistently with the presence of the vector sand-fly. Four cases had meningitis and two cases meningoencephalitis. Another case of meningitis was probably linked to Toscana virus based on positive serological results. These data confirm Toscana virus to be an emerging infection in the Mediterranean area, including our area. Three out of six patients were not born in Italy. Although figures are too little to allow any conclusion, we can speculate that possibly Toscana virus is more likely to induce severe clinical manifestations in non-immune adults and that it is probably quite spread in our communities. Many case reports in travelers and clinical research and epidemiologic studies conducted around the Mediterranean region have shown that Toscana virus has a tropism for the CNS and is a major cause of meningitis and encephalitis in countries in which it circulates. In central Italy, Toscana virus is the most frequent cause of meningitis from May to October, far exceeding enteroviruses. For instance, out of 120 cases of neuroinvasive infections, in Emilia Romagna in 2012, 28.3% of cases had Toscana virus antibodies and 79.4% of them were in the acute phase of the infection. [45] In other northern Mediterranean countries, TOSV is among the 3 most prevalent viruses associated with meningitis during the warm seasons. [46] Only in 1 out of four cases some kind of immunosuppression was present; in fact, differently by VZV and HSV-1, Toscana virus is not a typical opportunistic agent. In a series of 17 cases reported in France, All patients presented with fever and neurological signs were observed such as aseptic meningitis (n = 6), muscular symptoms (n = 3), or encephalitis (n = 4). The outcome was always favorable. At the acute stage, IgM were observed in 14/17 patients, neutralization tests were positive for 3/8 patients, and RT-PCR

confirmed Toscana virus infections in 5/8 CSF specimens. [47] Although Toscana virus infection is usually mild, severe encephalitis cases have been reported. [48]

No treatment options are currently available. A study on prevention from infection by Toscana virus reported that a combination of recombinant Toscana virus structural proteins N-Gc, used as a vaccine, protected 100% of mice infected with a lethal, neurovirulent strain of Toscana virus. [49] Other than prevention and antiviral therapy, repellents and insecticides are the principal options to reduce the spread of sandfly-borne diseases. Spraying campaigns are usually focused on inhabited areas and thus efficient against anthoponotic sandflies, such as *P. papatasi*. The efficacy is much lower against non-anthoponotic sandflies, such as those belonging to the Laroussius subgroup. However, without precise mapping of sandfly habitats and breeding areas, insecticide spraying is likely to be poorly effective. Because so little is known about natural breeding sites of sandflies, the preimaginal stages are rarely targeted by control measures. In campaigns against the adult sandflies, assessments of efficacy and cost/benefit are difficult to make because there are few properly controlled studies, and the results of different interventions are seldom compared. Insecticide spraying significantly decreases the incidence of *Phlebotomus*-transmitted diseases only if spraying is continuous; sporadic campaigns are considered to be ineffective. Several properties of the sandfly-borne phleboviruses make them good candidates for further emergence as human pathogens. Because the geographic distribution of these agents is dictated by the distribution of their vectors, climate change can modulate at-risk areas and human populations. The high rate of mutation of these viruses due to the lack of proofreading activity of the viral RNA polymerase generates quasispecies populations, a situation favoring the selection of variants with modified phenotypes, potentially including increased virulence and/or transmission efficiency. The

propensity for genetic reassortment or recombination under conditions of mixed infections may result in recombinant viruses with significantly altered pathogenicity characteristics, as has been observed with other genera in the *Bunyaviridae* family. [50, 51] By definition all arboviruses have the capacity to infect and replicate in both vertebrates and invertebrates. Thus, arboviruses have evolved the capability of infecting widely different hosts that present very distinct biochemical challenges. This "plasticity" in their life cycles increases their capacity to cross species barriers, an essential requirement for virus emergence. [52] Enteroviruses were responsible for one case of meningitis, one of meningoencephalitis and one of myelitis. All patients were immunocompetent and all of them fully recovered, consistently with the existing literature.

Two patients had West Nile virus IgM in the study period, consistently with the definition of probable case established by the European Centers for Disease Control. One patient had meningitis and one had myelitis. Both occurred in immunocompetent patients. Human cases of WNV-associated fever and/or neurological disorders have been reported in Italy since 2008. The first outbreak occurred in the northeastern region of Italy surrounding the Po River and was caused by the Po River lineage 1 strain, and since then, WNV infections have been reported in several regions of central Italy. Although the virus is highly genetically conserved, stochastic mutations in its genome may lead to the emergence of new strains, as was observed in Italy in 2011 with the identification of two new lineage 1 strains, the WNV Piave and WNV Livenza strains. [53] Confirmed human cases have been reported in Puglia, Lombardia, Veneto, Emilia Romagna and Sardinia Italian regions. [54] West Nile virus transmission has been confirmed in the last four years in Europe and in the Mediterranean Basin. An increasing concern towards West Nile disease has been observed due to the high number of human and animal cases reported in these areas confirming the importance of

this zoonosis. A new epidemiological scenario is currently emerging: although new introductions of the virus from abroad are always possible, confirming the epidemiological role played by migratory birds, the infection endemisation in some European territories today is a reality supported by the constant reoccurrence of the same strains across years in the same geographical areas. Despite the WND reoccurrence in the Old World, the overwintering mechanisms are not well known, and the role of local resident birds or mosquitoes in this context is poorly understood. A recent new epidemiological scenario is the spread of lineage 2 strains across European and Mediterranean countries in regions where lineage 1 strain is still circulating creating favourable conditions for genetic reassortment and emergence of new strains. [55]

In case symptoms of cerebral parenchyma involvement are present, VZV and HSV-1 were the most frequent etiology and should be considered in such a case; in case of pure meningitis, Toscana virus and HSV-2 were the most frequent etiology and should be considered in such a case.

Plain CT scan is not able to detect signs of meningitis or encephalitis and it can only be useful in ruling out contraindications to lumbar puncture. We believe at least in cases signs of encephalitis are evident at presentation magnetic resonance should be considered as first line examination.

EEG confirms its role in the diagnosis and follow-up of CNS infections.

An increased WBC count, particularly neutrophils, was present at admission in a relevant number of cases, and no concomitant bacterial infection was detected, meaning that this cannot be used as a criterion to discriminate between bacterial and viral infection of the CSF. On the opposite, procalcitonin seems to confirm its correlation with bacterial etiology.

An increased WBC count in CSF, usually lymphocytes, was present in almost all cases, confirming this to be a criterion to confirm or rule out meningitis. CSF/blood glucose level, more than absolute CSF glucose level, is a highly sensitive parameter to distinguish between aseptic and bacterial meningitis, also in line with recent reports. [56]

Mean WBC count was $285x10^9$/L, Enterovirus infection was associated with a mean WBC of $152x10^9$/L and VZV infection with a mean WBC of $625x10^9$/L. The fact that Enterovirus induced a mild inflammatory reaction in terms of leukocytes, is consistent with what already reported. [34] What is quite noteworthy is the significantly stronger inflammatory response, both in terms of leukocytes and proteins, induced by VZV in comparison with other etiologies in our case series.

The results of the seroprevalence survey confirm Toscana virus to be widely spread in our area. A wider seroprevalence survey conducted in the area of Siena found 19.8% of adults to be immunized against Toscana virus, with an age-dependent trend. [57] Other data indicate a seroprevalence of Toscana virus of 77.2% in the forestry workers of the Tuscany region. This fact is strictly correlated with the ecological niches specific for the survival of Toscana virus arthropod vector. [58]

Climate changes might impact on vectors activity and spread of emerging infections such as West Nile and Toscana virus infection.

Conclusions

VZV and TOSV are the most common causes of CNS viral infection in our setting. West Nile is an emerging cause of infection also in our area. VZV and HSV-1 are more frequently associated with brain parenchyma involvement.

An early lumbar puncture is necessary to increase the probability of obtaining an etiologic diagnosis. A high WBC count with a lymphocyte predominance, high or normal glucose levels and high proteins in CSF examination are suggestive of viral meningitis. Higher levels of WBC and proteins in CSF are suggestive of VZV infection while milder inflammatory response is suggestive of Enterovirus infection.

Procalcitonin levels may be useful in distinguishing between viral and bacterial meningitis while WBC count is not a sensible predictor.

Acknowledgments

References

1. Wan C et al. Viral meningitis. http://emedicine.medscape.com. Updated September 19, 2013

2. Rantakallio P, Leskinen M, Von Wendt L. Incidence and prognosis of central nervous system infections in a birth cohort of 12,000 children. Scand J Infect Dis 1986;18:287-94. [PubMed]

3. Davison KL, Ramsay ME. The epidemiology of acute meningitis in children in England and Wales. Arch Dis Child 2003;88:662-4. [PMC free article] [PubMed]

4. Kupila L, Vuorinen T, Vainionpää R, Hukkanen V, Marttila RJ, Kotilainen P. Etiology of aseptic meningitis and encephalitis in an adult population. Neurology 2006;66:75-80. [PubMed]

5. Sawyer MH, Rotbart H. Viral meningitis and aseptic meningitis syndrome. In: Scheld WM, Whitley RJ, Marra CM, eds. Infections of the central nervous system 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2004:75-93.

6. Chandy C. John, Hélène Carabin, Silvia M. Montano, Paul Bangirana, Joseph R. Zunt & Phillip K. Peterson, Global research priorities for infections that affect the nervous system, Nature 527, S178-186 (19 Nov 2015)

7. Huang CC, Liu CC, Chang YC et al. Neurologic complications in children with enterovirus 71 infection. N Engl J Med 1999;341:936–942.

8. Henquell C, Chambon M, Bailly JL et al. Prospective analysis of 61 cases of enteroviral meningitis: interest of systematic genome detection in cerebrospinal fluid irrespective of cytologic examination results. J Clin Virol 2001;21:29–35.

9. Dagan R, Jenista JA, Menegus MA. Association of clinical presentation, laboratory findings, and virus serotypes with the presence of meningitis in hospitalized infants with enterovirus infection. J Pediatr 1988;113:975–8.

10. Rotbart HA, Brennan PJ, Fife KH et al. Enterovirus meningitis in adults. Clin Infect Dis 1998;27:896–8.

11. Caro V, Guillot S, Delpeyroux F, Crainic R. Molecular strategy for 'serotyping' of human enteroviruses. J Gen Virol. 2001 Jan;82(Pt 1):79-91.

12. CDC. Final 2008 West Nile Virus Activity in the United States. Centers for Disease Control and Prevention. Available at http://bit.ly/fATcE1. Accessed September 1, 2009.

13. Berlin L.E., Rorabaugh M.L., Heldrich F., Roberts K., Doran T., Modlin J.F. Aseptic meningitis in infants <2 years of age: Diagnosis and etiology. J. Infect. Dis. 1993;168:888–892.

14. Pradier S, Lecollinet S, Leblond A. West Nile virus epidemiology and factors triggering change in its distribution in Europe. Rev Sci Tech. 2012;31(3):829–844 [PubMed]

15. Danis K, Papa A, Papanikolaou E, et al. Ongoing outbreak of West Nile virus infection in humans, Greece, July to Aug 2011. Euro Surveill. 2011;16(34):1–5. [PubMed]

16. European Centre for Disease Prevention and Control . Annual Epidemiological Report 2012 Reporting on 2010 Surveillance Data and 2011 Epidemic Intelligence Data. Stockholm: European Centre for Disease Prevention and Control; 2013.

17. ecdc.europa.eu [homepage on internet]Reported cases for West Nile fever for the EU and neighbouring countries: 2013 European Centre for Disease Prevention and Control; 2013 [cited December30, 2013]. Available from:

http://www.ecdc.europa.eu/en/healthtopics/west_nile_fever/west-nile-fever-maps/pages/index.aspxAccessed March 20, 2014

18. Charrel RN, Bichaud L, de Lamballerie X. Emergence of Toscana virus in the mediterranean area. World J Virol. 2012 Oct 12;1(5):135-41

19. Myung NS, Kim YJ, Kim YJ, Koo SK. Complicated mumps viral infection: An unusual presentation affecting only submandibular gland. Am J Otolaryngol. Apr 3 2013.

20. Hviid A, Rubin S, Mühlemann K. Mumps. Lancet. Mar 15 2008;371(9616):932-44.

21. Canada Communicable Disease Report. International Note: Global Advisory Committee on Vaccine Safety, 11-12 June, 2003. April 1, 2004.

22. Weekly Epidemiological Record. World Health Organization. January 19, 2007;No. 3, 2007, 82:18-22.

23. Landry ML, Greenwold J, Vikram HR. Herpes simplex type-2 meningitis: presentation and lack of standardized therapy. Am J Med. Jul 2009;122(7):688-91.

24. Johnson RT. Pathogenesis of CNS infections. In: Viral Infections of the Central Nervous System. New York: Raven; 1982:37-60.

25. Cassady KA, Whitley RJ. Pathogenesis and pathophysiology of viral infections of the central nervous system. In: Scheld WM, Whitley RJ, Marra CM, eds. Infections of the Central Nervous System. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2004:57-74.

26. Kanegaye JT, Nigrovic LE, Malley R, Cannavino CR, Schwab SH, Bennett JE, et al. Diagnostic value of immature neutrophils (bands) in the cerebrospinal fluid of children with cerebrospinal fluid pleocytosis. Pediatrics. Jun 2009;123(6):e967-71.

27. US Food and Drug Administration. FDA Clears Rapid Test for Meningitis. FDA. Available at http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108870 .htm. Accessed September 1, 2009.

28. King RL, Lorch SA, Cohen DM, Hodinka RL, Cohn KA, Shah SS. Routine cerebrospinal fluid enterovirus polymerase chain reaction testing reduces hospitalization and antibiotic use for infants 90 days of age or younger. Pediatrics. Sep 2007;120(3):489-96. [Medline].

29. Marque Juillet S, Lion M, Pilmis B, Tomini E, Dommergues MA, Laporte S, et al. [Value of polymerase chain reaction in serum for the diagnosis of enteroviral meningitis.]. Arch Pediatr. Apr 26 2013;[Medline].

30. Kleines M, Scheithauer S, Schiefer J, Häusler M. Clinical application of viral cerebrospinal fluid PCR testing for diagnosis of central nervous system disorders: a retrospective 11-year experience. Diagn Microbiol Infect Dis. 2014 Nov;80(3):207-15.

31. Granerod J, Ambrose HE, Davies NW, Clewley JP, Walsh AL, Morgan D, Cunningham R, Zuckerman M, Mutton KJ, Solomon T, Ward KN, Lunn MP, Irani SR, Vincent A, Brown DW, Crowcroft NS; UK Health Protection Agency (HPA) Aetiology of Encephalitis Study Group. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. Lancet Infect Dis. 2010 Dec;10(12):835-44.

32. Akhvlediani T, Bautista CT, Shakarishvili R, Tsertsvadze T, Imnadze P, Tatishvili N, Davitashvili T, Samkharadze T, Chlikadze R, Dvali N, Dzigua L, Karchava M, Gatserelia L, Macharashvili N, Kvirkvelia N, Habashy EE, Farrell M, Rowlinson E,

Sejvar J, Hepburn M, Pimentel G, Dueger E, House B, Rivard R. Etiologic Agents of Central Nervous System Infections among Febrile Hospitalized Patients in the Country of Georgia. PLoS One. 2014 Nov 4;9(11):e111393.

33. Lautaret S, Gennai S, Sellier E, Wintenberger C, François P, Carpentier F, Pavese P. Suspicion of meningitis: evaluation of the management in the emergency unit. Presse Med. 2013 Mar;42(3):e69-77.

34. Ihekwaba UK, Kudesia G, McKendrick MW. Clinical features of viral meningitis in adults: significant differences in cerebrospinal fluid findings among herpes simplex virus, varicella zoster virus, and enterovirus infections. Clin Infect Dis. 2008 Sep 15;47(6):783-9.

35. Chaves SS, Haber P, Walton K, Wise RP, Izurieta HS, Schmid DS, Seward JF. Safety of varicella vaccine after licensure in the United States: experience from reports to the vaccine adverse event reporting system, 1995-2005.

36. Science M, MacGregor D, Richardson SE, Mahant S, Tran D, Bitnun A. Central nervous system complications of varicella-zoster virus. J Pediatr. 2014 Oct;165(4):779-85.

37. Pahud BA, Glaser CA, Dekker CL, Arvin AM, Schmid DS. Varicella zoster disease of the central nervous system: epidemiological, clinical, and laboratory features 10 years after the introduction of the varicella vaccine. J Infect Dis. 2011 Feb 1;203(3):316-23.

38. Iyer S, Mittal MK, Hodinka RL. Herpes zoster and meningitis resulting from reactivation of varicella vaccine virus in an immunocompetent child. Ann Emerg Med. 2009 Jun;53(6):792-5.

39. Levin MJ, DeBiasi RL, Bostik V, Schmid DS. Herpes zoster with skin lesions and meningitis caused by 2 different genotypes of the Oka varicella-zoster virus vaccine. J Infect Dis. 2008 Nov 15;198(10):1444-7.

40. Han JY, Hanson DC, Way SS. Herpes zoster and meningitis due to reactivation of varicella vaccine virus in an immunocompetent child. Pediatr Infect Dis J. 2011 Mar;30(3):266-8.

41. Franco E, Gabutti G, Bonanni P, Conversano M, Stefano Valente ME, Ferro A, Icardi G, Antonio Volpi ML, Maggi S, Rossi A, Scotti S, Vitale F, Greco D; Italian Universities and Epidemiology. Franco E, Gabutti G, Bonanni P, Conversano M, Stefano Valente ME, Ferro A, Icardi G, Antonio Volpi ML, Maggi S, Rossi A, Scotti S, Vitale F, Greco D; Italian Universities and Epidemiol J Infect Dis. 2008 Mar 1;197 Suppl 2:S170-7.

42. Fusco D, Krawitz P, LaRussa P, Steinberg S, Gershon A, Jacobs J. VZV meningitis following varicella vaccine. J Clin Virol. 2010 Aug;48(4):275-7.

43. Piret J, Boivin G. Antiviral drug resistance in herpesviruses other than cytomegalovirus. Rev Med Virol. 2014 May;24(3):186-218.

44. van Velzen M, van de Vijver DA, van Loenen FB, Osterhaus AD, Remeijer L, Verjans GM. Acyclovir prophylaxis predisposes to antiviral-resistant recurrent herpetic keratitis. J Infect Dis. 2013 Nov 1;208(9):1359-65.

45. Pierro A, Landini MP, Gaibani P, Rossini G, Vocale C, Finarelli AC, Cagarelli R, Sambri V, Varani S. A model of laboratory surveillance for neuro-arbovirosis applied during 2012 in the Emilia-Romagna region, Italy.Clin Microbiol Infect. 2014 Jul;20(7):672-7.

46. Charrel RN, Gallian P, Navarro-Mari JM, Nicoletti L, Papa A, Sánchez-Seco MP, Tenorio A, de Lamballerie X. Emergence of Toscana virus in Europe. Emerg Infect Dis. 2005 Nov;11(11):1657-63.

47. Dupouey J, Bichaud L, Ninove L, Zandotti C, Thirion-Perrier L, de Lamballerie X, Charrel RN. Toscana virus infections: a case series from France. J Infect. 2014 Mar;68(3):290-5.

48. Papa A, Paraforou T, Papakonstantinou I, Pagdatoglou K, Kontana A, Koukoubani T. Severe encephalitis caused by Toscana virus, Greece. Emerg Infect Dis. 2014 Aug;20(8):1417-9.

49. Gori Savellini G, Di Genova G, Terrosi C, Di Bonito P, Giorgi C, Valentini M, Docquier JD, Cusi MG. Immunization with Toscana virus N-Gc proteins protects mice against virus challenge. Virology. 2008 Jun 5;375(2):521-8.

50. Bowen MD, Trappier SG, Sanchez AJ, Meyer RF, Goldsmith CS, Zaki SR, Dunster LM, Peters CJ, Ksiazek TG, Nichol ST; RVF Task Force. A reassortant bunyavirus isolated from acute hemorrhagic fever cases in Kenya and Somalia. Virology. 2001 Dec 20;291(2):185-90.

51. Gerrard SR1, Li L, Barrett AD, Nichol ST. Ngari virus is a Bunyamwera virus reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa. J Virol. 2004 Aug;78(16):8922-6.

52. Elliott RM, Bouloy M, Calisher CH, Goldbach R, Moyer JT, Nichol ST, Pettersson R, Plyusnin A, Schmaljohn CS. Family *Bunyaviridae* in Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego (2000) pp. 599–621.

53. Delbue S, Ferrante P, Mariotto S, Zanusso G, Pavone A, Chinaglia M, L'Erario R, Monaco S, Ferrari S. Review of West Nile virus epidemiology in Italy and report of a case of West Nile virus encephalitis. J Neurovirol. 2014 Oct;20(5):437-41.

54. Pupella S, Pisani G, Cristiano K, Catalano L, Grazzini G. Update on West Nile virus in Italy. Blood Transfus. 2014 Oct;12(4):626-7.

55. Di Sabatino D, Bruno R, Sauro F, Danzetta ML, Cito F, Iannetti S, Narcisi V, De Massis F, Calistri P. Epidemiology of West Nile disease in Europe and in the Mediterranean Basin from 2009 to 2013. Biomed Res Int. 2014;2014:907852.

56. Tamune H, Takeya H, Suzuki W, Tagashira Y, Kuki T, Honda H, Nakamura M. Cerebrospinal fluid/blood glucose ratio as an indicator for bacterial meningitis. Am J Emerg Med. 2014 Mar;32(3):263-6.

57. Terrosi, C., Olivieri, R., Bianco, C., Cellesi, C., Cusi, M.G., 2009. Age-dependent seroprevalence of Toscana virus in central Italy and correlation with the clinical profile. Clin. Vaccine Immunol. 16, 1251–1252.

58. Valassina M, Valentini M, Pugliese A, Valensin PE, Cusi MG. Serological survey of Toscana virus infections in a high-risk population in Italy. Clin Diagn Lab Immunol. 2003 May;10(3):483-4.