

Update of microbiological diagnosis of CNS viral meningitis and encephalitis

Corinne Liesnard

November 2011

- Lumbar puncture: to confirm (or exclude) a bacterial infection.
- Why a diagnosis of a CNS viral infection?
 - prognostic information
 - patient care
 - diminution of antibiotics use
 - diminution of hospitalisation time
 - measures to prevent spread of infection

How common is viral meningitis ?

- Frequent in young children.
- Finland: 12.000 children (birth cohort)
annual incidence of viral meningitis:

219/100.000 (< 1 year)

27.8/100.000 (< 14 years)

7.6/100.000 (> 16 years)

Viral meningitis is notifiable (England)

2005-2006: 2898 patients admitted to
hospital with a diagnosis of viral meningitis:

10 X the number of notified cases

Viruses in meningitis

- Enterovirus: in children, first cause of viral meningitis
- Adults: 144 cases of aseptic meningitis
72 (1/2) with a diagnosis:
 - EV: 45%
 - HSV2: 31.5%
 - VZV: 11%
 - HSV1: 4%

Clinical Diagnosis

- No typical symptomatology of viral or bacterial meningitis: acute onset with fever, headache, photophobia, neck stiffness, nausea, vomiting...
 - deterioration of mental status in patient with bacterial meningitis
 - spontaneous recovery in case of viral infection
 - no specific indicator: all cases referred to hospital

Clinical diagnosis

- Neonates and young children: fever, irritability, seizures,
- Neonates: signs of meningeal inflammation (fontanella bulging)
- Systemic involvement:
 - hepatic necrosis
 - myocarditis
 - necrotizing enterocolitis

Viral causes of encephalitis and meningitis

- Etiology of encephalitis and meningitis:
unknown in most cases!

Importance of the diagnosis: therapy?

2357 CSF from patients with encephalitis or meningitis

340 CSF + (14.8%): enterovirus: 5.5%

EBV 3.6 %

HSV1 and 2 2.8%

VZV 1.9%

Diagnosis of herpesvirus infection of the CNS

- Guidelines from the International Herpes Management Forum (2004):
 - use of PCR analysis of CSF for the diagnosis of HSV, VZV and CMV infections of the CNS
 - Participation to a proficiency testing program
 - For retrospective diagnosis, use measure of the intrathecal antibody production
 - Viral culture is not recommended (except in suspected neonatal HSV infection)
 - In case of suspicion of CMV myelitis, PCR and viral culture could be used for diagnosis.
 - PCR analysis of CSF for EBV can help for the diagnosis of AIDS associated primary CNS lymphoma

Herpesviruses cause various CNS and PNS disorders in children and adults immunodeficient or not

- HSV1, HSV2, VZV, CMV: encephalitis
- VZV, CMV, EBV, HSV2: myelitis
- VZV, HSV2: acute meningitis
- VZV: ventriculitis, herpes zoster ophthalmicus
- HSV2: recurrent meningitis
- CMV: polymyeloradiculonevritis
- HHV6: seizures

Viral meningitis

- N of viral meningitis >> N of meningitis of other etiology.
- Aseptic meningitis = clinical syndrome:
 - meningeal inflammation
 - no bacterial pathogen
 - no encephalitis, no myelitis
 - benign course
 - also in the setting of an underlying disorder, malignancy, following drug administration,...

Clinical features of viral meningitis

- Asymptomatic pleiocytosis in the CSF < > neurological impairment (usually fever, headache, stiff neck, malaise, anorexia, vomiting).

Neonates: signs of meningeal inflammation, fontanel bulging,...

systemic involvement: hepatic necrosis

myocarditis

necrotizing enterocolitis

Herpetic encephalitis diagnosis

CSF: HSV culture: positive in < 5% of cases!

HSV PCR: sensitivity: 98% /positive biopsies

specificity: 94%/ negative biopsies

PPV: 95% NPV: 98%

Neonates: sensitivity: 75 %

specificity: 100%

PPV: 100% NPV: 98%

HSV PCR inhibition

- 164 CSF + 200 copies HSV: 1 % inhibition
 - 1 xanthochromic sample
 - 1 sample + red cells hemolysis

Kinetics of the PCR positivity

- PCR can be negative during the first 72H of infection. Do not stop the treatment and repeat the PCR between day 4 and day 7.
- Effect of antiviral therapy (Acyclovir):
HSV detectable for 5 days at least – decline after 2 weeks and undetectable after 30 days.
If the PCR is positive after 2 weeks' treatment, additional treatment course

Neonatal Herpes

- Viral load quantification → prognostic determination
- Disseminated infection: high viral load in the serum
- CNS infection: high viral load in the CSF
- Association of high viral load with poor prognosis
- HSV2 infected patients have higher viral load than HSV1 infected patients, with more CNS damage and handicap.
- In the CSF: disseminated infection: HSV DNA: 93%
 - CNS infection: 76%
 - hand-mouth-eyes disease: 24%

Enteroviruses

First cause of viral meningitis in children (and in adults)

Broad spectrum of CNS diseases:

- « benign » aseptic meningitis: 75000 cases and 50000 hospitalisations/year in the USA.
- EV encephalitis: local or diffuse process, third cause of encephalitis (after HSV and arboviroses)
- Severe sepsis syndrome in newborns: meningo-encephalitis, myocarditis, hepatitis

Diagnosis of Enterovirus infections

- Rapid detection using RT-PCR (5' non translated but well-conserved region of EV)
high sensitivity: 96-100% and specificity: 96%
- Viral culture: sensitivity: 65-75% (low viral load, some EV are not cultivable)
- Prolonged excretion: oropharynx (4 weeks), gastrointestinal tract: 16 weeks
- Up to 50% of children <1 month of age with illness compatible with EV infection, have no CSF pleiocytosis, but detectable EV in the CSF.

Enterovirus: impact on management

- EV PCR before hospital discharge: 95 positives, 92 negatives (!)
- Reduction in length hospital stay, time of discharge, number of tests performed, days of IV antibiotics.
- 59 % reduction of hospital costs (infants < 6 months of age)
- Costs saving estimation: 2000 dollars/ patient

Good to know about CSF and enteroviruses

- Cytologic analysis of the CSF has no predictive value. There is a predominance of polynuclear cells in 50% of the enterovirus cases.
- No pleiocytosis in the CSF: up to 18% of the CSF.
- Neonatal sepsis, transverse myelitis, encephalitis are caused by enterovirus

Good to know about enterovirus

- Management of patients suspected of EV meningitis:
- If PCR is positive →
- immediate discharge (2H30) of 63 % of the children.
- within 24H discharge: 95% of children
- 2 days discharge: infants and adults

EBV

- Immunocompetent: (adults seropositivity: 90%)

aseptic meningitis, encephalitis, cerebellar ataxia, myelitis,...

Normally no detection of EBV in the CSF (even in case of another infection in the CNS).

Diagnosis: EBV PCR on CSF

- Immunocompromised: AIDS-related CNS lymphoma. The EBV viral load is predictive of the CNS involvement

EBV

- In AIDS patients with an AIDS-related non Hodgkin lymphoma of the CNS or a primary lymphoma of the CNS and in control HIV positive patients without CNS involvement:

EBV detected in

67% of CNS lymphoma

80% of primary CNS lymphoma

32 % of systemic lymphoma

13 % of controls

EBV PCR could help to follow treatment efficacy

Diagnostic tardif d'une infection du SNC (encéphalites)

Démonstration de la production intrathécale d'anticorps spécifiques:

protéines du LCR < effraction de la barrière hémato-méningée (albumine, IgG)

<production locale d'IgG

en cas de barrière H-M altérée, albumine et IgG ↗ et le rapport entre les 2 reste constant

◆ Barrière hémato-méningée normale:

Alb LCR/Alb sérum < 0.009 (âge dépendant)

◆ Index IgG/Alb normal:

IgG LCR/IgG sérum < 0.7

Alb LCR/ Alb sérum

Alb LCR/Alb sérum > 0.009 → barrière H-M
altérée → Index IgG/Alb ↗

Alb LCR/Alb sérum < 0.009 et Index IgG/Alb > 0.7



production intrathécale d'IgG

Lorsque des anticorps spécifiques (ex: IgG anti-HSV) sont détectés dans le LCR → production locale ou passage passif ?

1. Vérifier l'intégrité de la barrière H-M par le rapport Alb LCR/Alb sérum (<0.009)

2. Calculer l'index d'anticorps spécifiques:

Ac HSV(quantitatif) LCR/Ac HSV sérum <1.91

Alb LCR/Alb sérum

Si index Ac spécifiques >1.91 et barrière H-M normale → production intrathécale de ces anticorps démontrant une réponse immune locale vis à vis de l'infection.

Diagnosis of viral meningitis and encephalitis

What and When to order ?

CSF



early (first days of symptoms)

- ♦ **Virus detection by nucleic acid amplification tests**
Enterovirus (80% sensitivity)
HSV (95% sensitivity)
- ♦ **Culture :**
Enterovirus (60% sensitivity)
HSV (4% sensitivity)

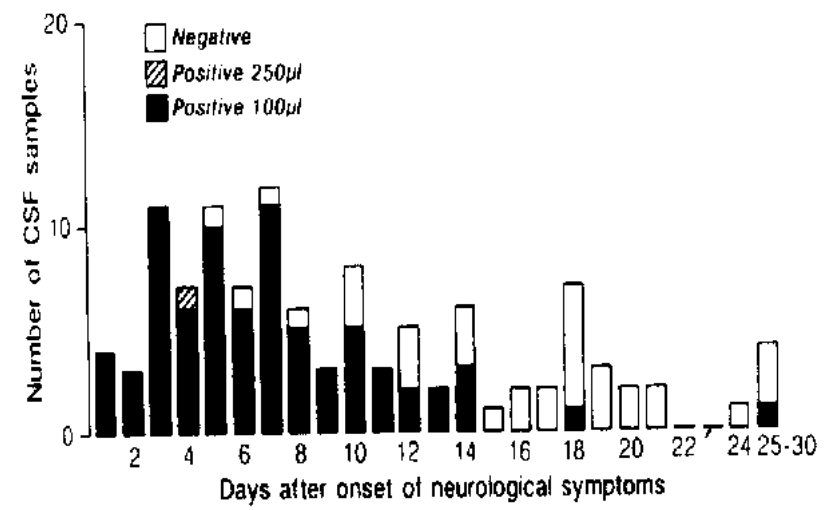
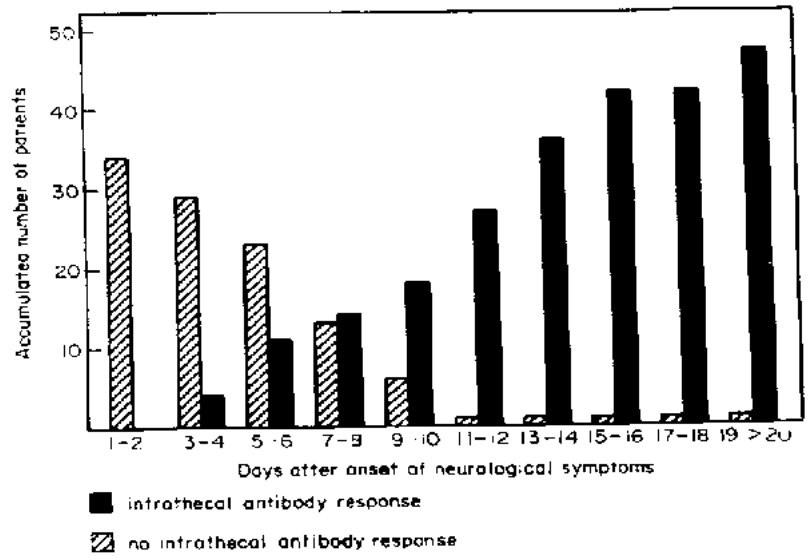
late (2-3 weeks)

- ♦ **Virus specific antibody intrathecal synthesis**
HSV (80% sensitivity)
Enterovirus (serology not available)
Do not forget a blood sample !

but also : stool sample
throat swab
urine

for viral culture : enterovirus
mumps

Herpes simplex encephalitis: Intrathecal antibody production and PCR results



Prevalence of viruses detected in the CSF by PCR (1995-2001)

Real time PCR

Virus N of PCR + in CSF samples (576)

| | | |
|------------|-----------|---------------------|
| EV | 409 (71%) | |
| HSV1 | 54 (9,4%) | |
| CMV | 41 (7,1%) | |
| VZV | 29 (5%) | 26% = Herpesviruses |
| HSV2 | 13 (2,3%) | |
| JC virus | 12 (2,1%) | |
| EBV | 11 (1,9%) | |
| HHV6 | 5 (0,9%) | |
| Parvovirus | 2 (0,3%) | |

↗ diagnosis by testing for different viruses

↗ in VZV diagnosis

Aberle, 2003

Table 1 Etiology of aseptic meningitis

| Etiology | Confirmed | Probable | Possible | Total, no. (%) |
|-----------------------|-----------|----------|----------|-------------------|
| Enterovirus | 33 | 5 | | 38 (26) |
| HSV-2 | 22 | 2 | | 24 (17) |
| VZV | 8 | 4 | | 12 (8) |
| TBEV | 2 | 6 | | 8 (6) |
| HSV-1* | 3 | | | 3 (2) |
| Other defined agents† | 4 | 4 | 2 | 10 (7) |
| Undefined agents | | | | 49 (34) |

* Both HSV-1 and VZV etiologies in one patient.

† EBV (n = 2), *M pneumoniae* (n = 2), *B burgdorferii* (n = 2), adenovirus (n = 1), parainfluenzavirus (n = 1), *T gondii* (n = 1), and trimethoprim (n = 1).

HSV = herpes simplex virus; VZV = varicella zoster virus;
TBEV = tick-borne encephalitis virus.

Etiology of meningitis in Finland (2006)

- PCR
- Antibodies to virus, mycop., chlam., borrelia
- CSF, throat and fecal swabs for viral culture

Aseptic meningitis : 66% etiology

EV : 26% > HSV2 : 17% (25% women) > VZV: 8%

Encephalitis : 36% etiology

VZV : 12% > HSV1 : 9%, TBE : 9%

PCR : + in 45% of aseptic meningitis

+ in 17% of encephalitis

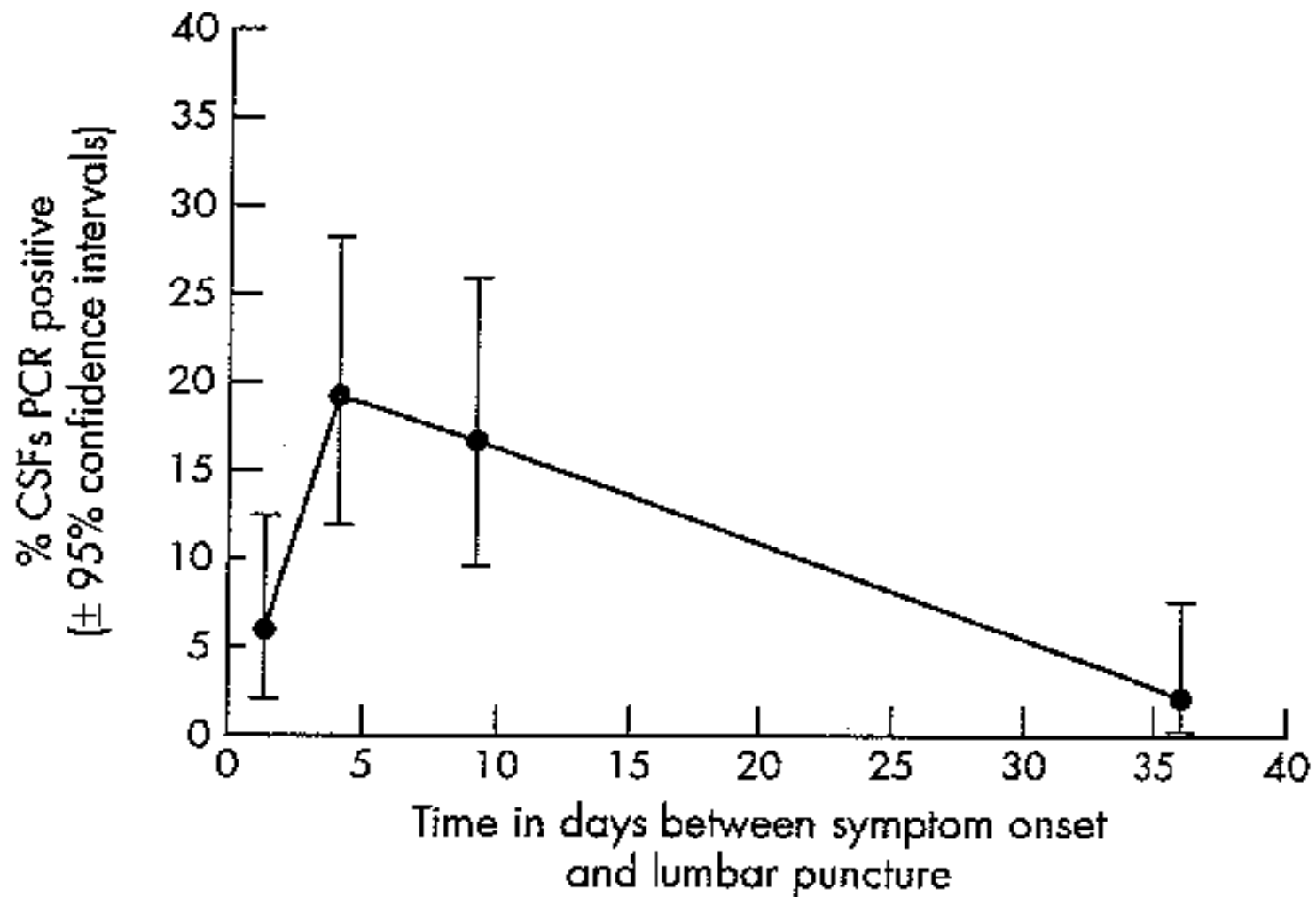


Figure 1 The relationship of virus detection by PCR with time delay between onset of neurological symptoms and lumbar puncture. *Numbers of PCR positive and negative CSF samples in each group are found in table 3.