

Acute Infectious Encephalitis Challenges in Clinical and Biological Diagnosis

**The Value of New Tools and Trusted Recipes
In the Etiological Diagnosis of Viral encephalitis :**

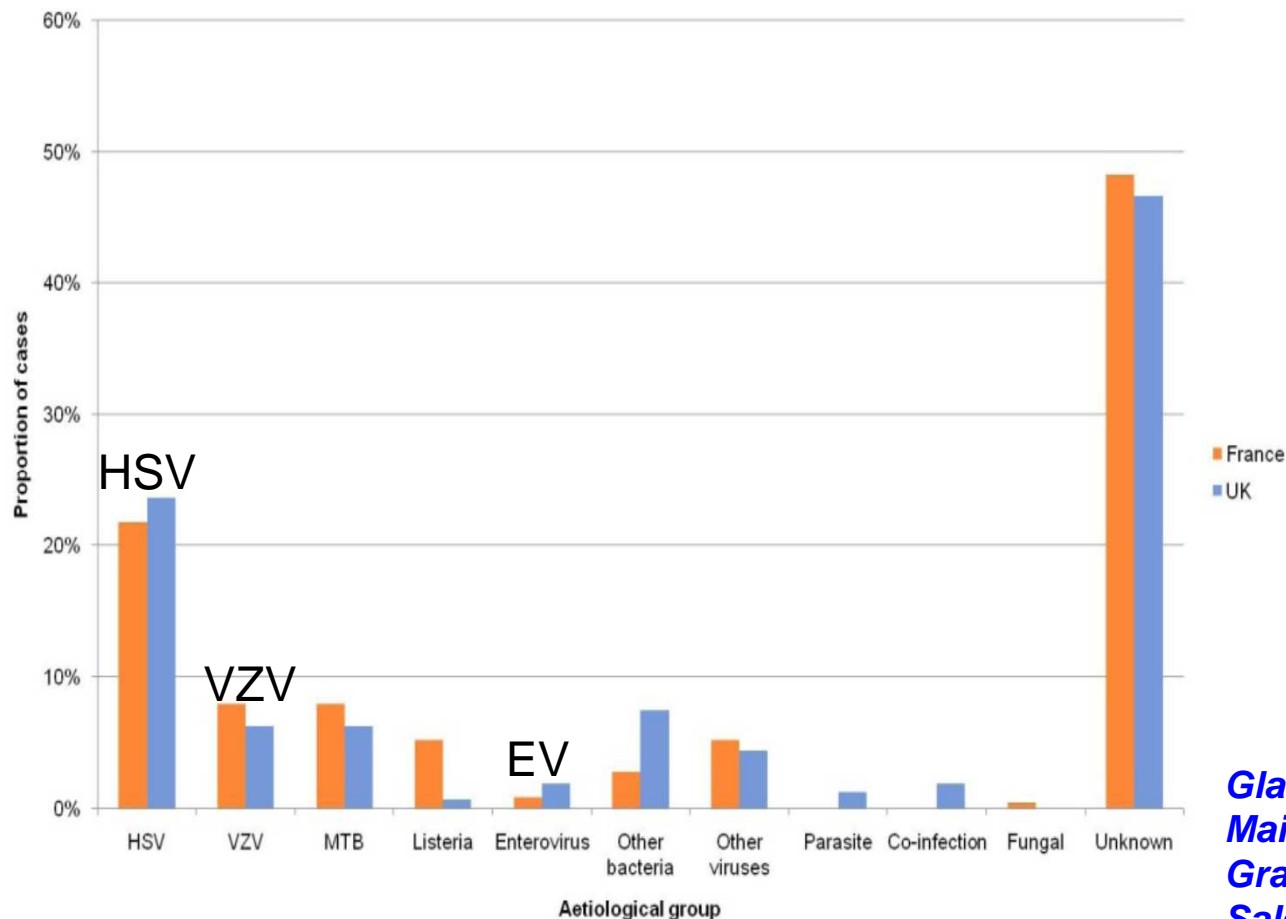
« A Virologist's Point of View »

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ESCMID Course Grenoble, October 2014
(no conflict of interest)



Acute encephalitis : Infection is still the most commonly demonstrated pathogenic mechanism

- . $\approx 50\%$ of cases are due to infectious agents (mainly viruses)
- . demonstrated ? = confirmed / probable / possible
- . importance of molecular methods (real time PCR) in CSF
- . serology still useful (intrathecal synthesis, serum)



*Glaser C, Clin Infect Dis 2006,
Mailles A, Clin Infect Dis 2009
Granerod J, Lancet infect Dis, 2011
Salomon T J Infect 2012*

Diagnostic Algorithm for Initial Evaluation of Encephalitis in Adults^a

ROUTINE STUDIES

CSF

Collect at least 20 cc fluid, if possible; freeze at least 5–10 cc fluid, if possible

Opening pressure, WBC count with differential, RBC count, protein, glucose

Gram stain and bacterial culture

HSV-1/2 PCR (if test available, consider HSV CSF IgG and IgM in addition)

VZV PCR (sensitivity may be low; if test available, consider VZV CSF IgG and IgM in addition)

Enterovirus PCR

Cryptococcal antigen and/or India Ink staining

Oligoclonal bands and IgG index

VDRL

SERUM

Routine blood cultures

HIV serology (consider RNA)

Treponemal testing (RPR, specific treponemal test)

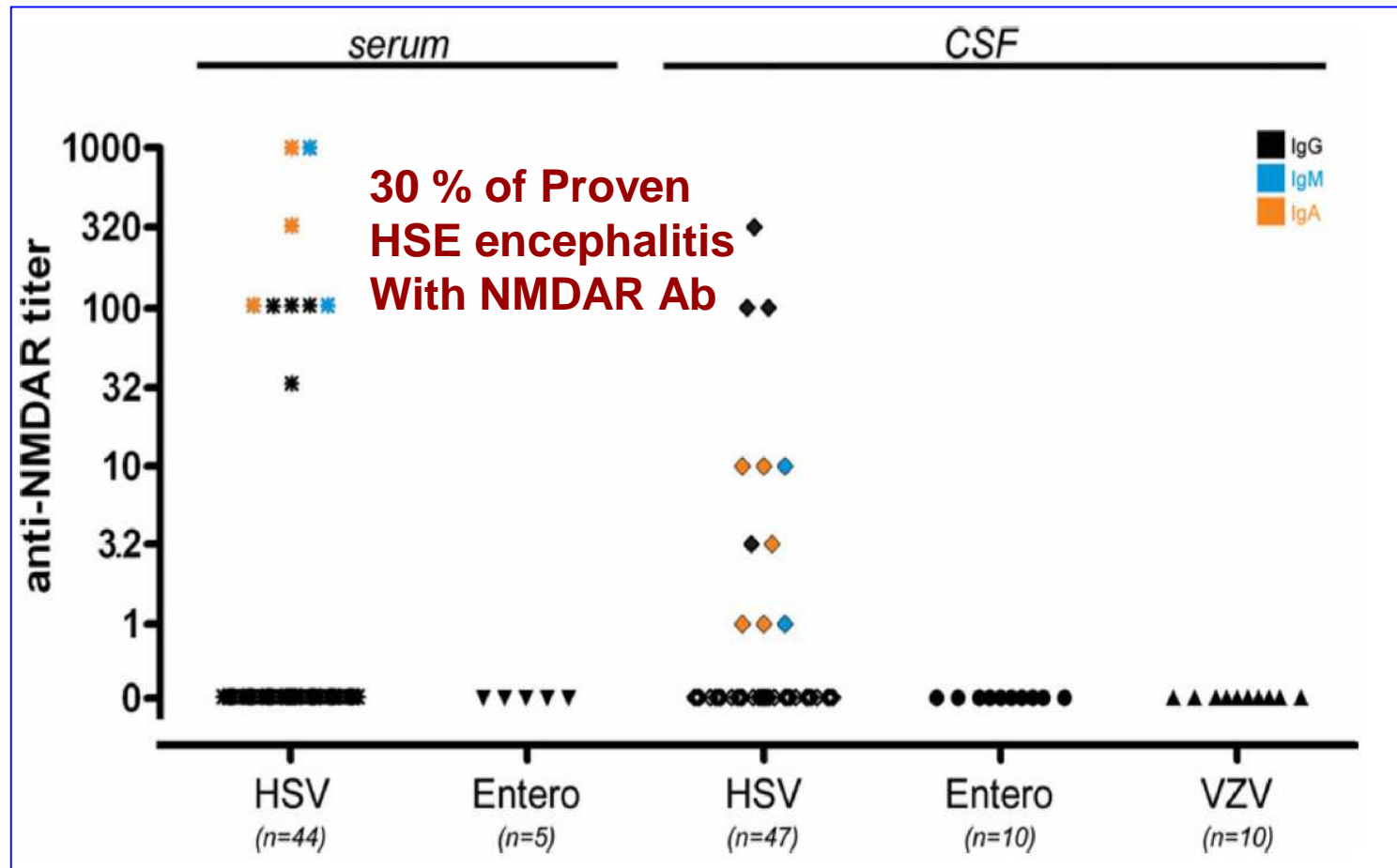
Hold acute serum and collect convalescent serum 10–14 d later for paired antibody testing

+ **Conditionnal studies :**

Host Factors / Geographic Factors / Season and exposure/ Specifics signs

Acute encephalitis :
Increasing description of autoimmune encephalitis
new pathophysiological concepts between virus and autoimmunity

HSV could be involved in autoimmune encephalitis



Acute encephalitis :

Increasing description of autoimmune encephalitis

What is the real frequency of autoimmune encephalitis ?

The Frequency of Autoimmune N-Methyl-D-Aspartate Receptor Encephalitis Surpasses That of Individual Viral Etiologies in Young Individuals Enrolled in the California Encephalitis Project *Gable M, Clin Infect Dis 2012*

761 encephalitis < 30 years
“diagnostically challenging”

→ **42 viral causes**
(30 entero, 7HSV, 5 WNV)

→ **32 NMDAR encephalitis**

Autoimmune N-methyl-D-aspartate receptor encephalitis is a differential diagnosis of infectious encephalitis

Thomas L, J Infect 2012

108 encephalitis
(6mo-88years median 55)

→ **38 infectious causes**

→ **Autoantibody screening :**
. 2 NMDAR (1.8%)
13 and 77 year old
.No MDR in the 17
HSV encephalitis

Acute encephalitis :

Still 40- 50 % of acute encephalitis with no clear etiological diagnosis in spite of new molecular tools :

Diagnostic Strategy Used To Establish Etiologies of Encephalitis in a Prospective Cohort of Patients in England^v

“.... Viral metagenomic studies using next-generation sequencing were undertaken to try to identify any novel pathogens present in 36 samples with no other etiology . Although no new viral sequences were identified, the possibility of their presence at a low titer in some of the samples cannot be excluded.

Ambrose H, J Clin Microbiol 2011



PCR is the gold standard for the diagnosis of CNS Viral Infections

Table 1 Recommendations for the use of PCR for the diagnosis of CNS viral infections

Virus	Reported sensitivity and specificity of CSF PCR	Evidence class and level of recommendation
Herpes simplex virus (HSV)-1 Encephalitis	96% and 99% [15]	Class I Level A May be false negatives during first 3 days
Varicella-Zoster virus (VZV)	80% and 98% [25]	Class III Level C CSF anti-VZV IgG more sensitive than PCR in VZV vasculopathy
Cytomegalovirus (CMV)	92% and 94% [32]	Class II Level B Quantitative PCR may also be clinically useful
Epstein-Barr Virus (EBV)	97-100% and 98.5% [33,34,36]	Class IV Level C Quantitative PCR may also be clinically useful
Enteroviruses	31-95% and 92-100% [37,40, 41]	Class II Level B
JC virus (JCV)	50-82% and 98.5-100% [48-50]	Class II Level B Quantitative PCR may also be clinically useful
Human immunodeficiency virus (HIV)	Diagnosis will already have been made on the blood	CSF viral load a useful tool in assessing neurological involvement
Human T-cell lymphotropic Virus (HTLV-1)	75-99.4% and 98.5% [40,57]	Class III Level C Combination of CSF PCR and anti-HTLV-1 antibody index useful in diagnosis

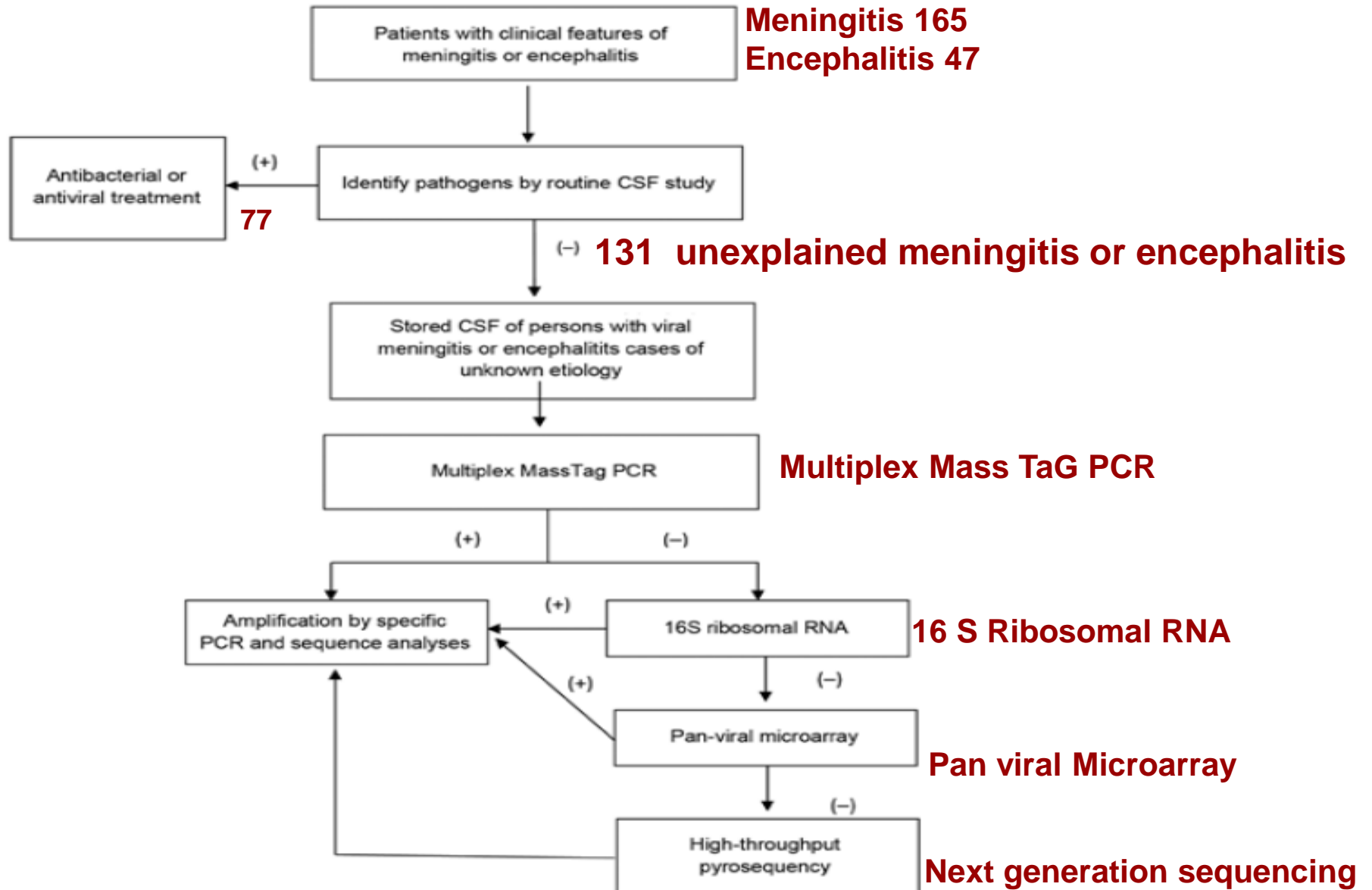
PCR is the gold standard :
What Else ?

Virological Diagnosis of Central Nervous System Infections by Use of PCR Coupled with Mass Spectrometry Analysis of Cerebrospinal Fluid Samples
Leveque N, J Clin Microbiol 2014

TABLE 4 Comparison of neurotropic virus detection in CSF samples using routine PCR and PCR-MS assays^a

PCR-MS result	No. of samples with indicated result in routine PCR assay													
	HSV		VZV		CMV		EBV		HHV-6		JCV		Enterovirus	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Pos	23	8 ^c	15	4 ^e	1	0	8	8 ^g	6	5 ^h	5	1 ⁱ	129	1 ^k
Neg	2 ^b	294	1 ^d	307	0	326	18 ^f	293	0	316	0	321	25 ^j	172
Kappa test (95% CI)	0.80 (0.69–0.92)		0.85 (0.71–0.98)		NC		0.34 (0.10–0.58)		NC		NC		0.84 (0.78–0.90)	
Sensitivity (%)	92		94		NC		31		NC		NC		84	
Specificity (%)	97		99		NC		97		NC		NC		99	

PCR is the gold standard :
What Else ? : Use of staged molecular analysis



. Multiplex Mass tag PCR + 16 s rRNA + MicroArray :

→ **31 new diagnosis (23%) / 131**

. After Multiplex Mass tag PCR + 16 s rRNA + MicroArray :

→ **no need of next generation sequencing**

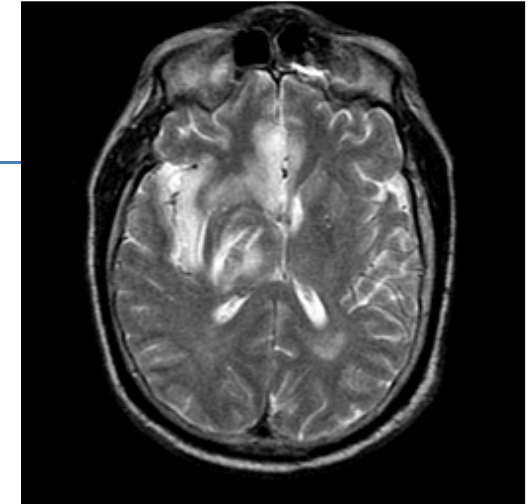
Table 3. Pathogens identified by a staged molecular approach

Pathogen	No. (%) cases			No. HIV positive	Molecular method
	Total	Meningitis	Encephalitis		
Virus					
Enterovirus*	2 (6)	2 (8)	0	0	MassTag PCR
Human herpesvirus 1	2 (6)	0	2 (33)	← 0	MassTag PCR/microarray†
Human herpesvirus 2	1 (3)	1 (4)	0	0	MassTag PCR
Varicella-zoster virus	3 (10)	0	3 (50)	← 0	MassTag PCR
Epstein-Barr virus	16 (52)	→ ? 16 (64)	0	9	MassTag PCR
Cytomegalovirus	1 (3)	0	1 (17)	0	MassTag PCR
Bacteria					
<i>Mycobacterium tuberculosis</i>	1 (3)	1 (4)	0	0	MassTag PCR
<i>Escherichia coli</i>	5 (16)	5 (20)	0	1	16S rRNA PCR
Total	→ 31 (100)	25 (100)	→ 6 (100)	10	

*Sequences of enteroviruses showed 1 infection each of echovirus 11 and echovirus 30.

†For 1 case, human herpesvirus 1 was identified by MassTag PCR; in the other, by DNA microarray.

Are there some pitfalls/ limits with HSV PCR during Herpes simplex encephalitis ?



PCR in CSF : the gold standard since 1995 :

- . Sensitivities 96-98 % (less in children ?)
- . Specificities 95- 99 %
- . Positive predictive values \approx 100 %
- . Negative predictive values \approx 98%

└─→ **PCR negative and clinical suspicion of HSE :**

- **Maintain ACV**
- **Perform a second HSV PCR at day 3 - 7 : if neg stop acycl.**

Quality Controls Molecular Diagnostics 2010 (216 participants / 32 countries)

Table 3: Number of correct qualitative results per panel member and technology type

Sample	Sample content	Sample conc. Copies/ml	Total datasets n=227		PCR				NASBA ^e					
					Conventional		Real time		n=5					
					Commercial ^a n=9	In-house ^b n=13	Commercial ^c n=79	In-house ^d n=121						
n	%	n	%	n	%	n	%	n	%					
HSVDNA10-07	HSV-1	9,016	223	98.2	9	100.0	12	92.3	77	97.5	120	99.2	5	100
HSVDNA10-02	HSV-1	968	218	96.0	9	100.0	11	84.6	75	94.9	118	97.5	5	100
HSVDNA10-06	HSV-1	129	170	74.9	5	55.6	7	53.8	61	77.2	94	77.7	3	60
HSVDNA10-10	HSV-1	116	173	76.2	6	66.7	6	46.2	62	78.5	96	79.3	3	60
HSVDNA10-01	HSV-2	4,710	222	97.8	9	100.0	13	100.0	76	96.2	119	98.3	5	100
HSVDNA10-05	HSV-2	1,294	217	95.6	8	88.9	11	84.6	75	94.9	118	97.5	5	100
HSVDNA10-09	HSV-2	178	175	77.1	5	55.6	8	61.5	55	69.6	103	85.1	4	80
HSVDNA10-03	HSV-2	69	58	25.6	1	11.1	4	30.8	18	22.8	35	28.9	0	0
HSVDNA10-04	VZV		223	98.2	9	100.0	11	84.6	79	100.0	119	98.3	5	100
HSVDNA10-08	Negative		221	97.4	9	100.0	13	100.0	77	97.5	117	96.7	5	100

False negative with low viral load HSV 1 ≈ 22 %

False negative with low viral load HSV2 ≈ 77 % !!

False Positive ≈ 2.5 %

Herpes Simplex Encephalitis with Two False-Negative Cerebrospinal Fluid PCR Tests and Review of Negative PCR Results in the Clinical Setting

Adler A, Case Rep Neurol 2011

The NEW ENGLAND JOURNAL of MEDICINE

CLINICAL PROBLEM-SOLVING

Caren G. Solomon, M.D., M.P.H., *Editor*

A Creeping Suspicion

Rice C, N Engl J Med 2014

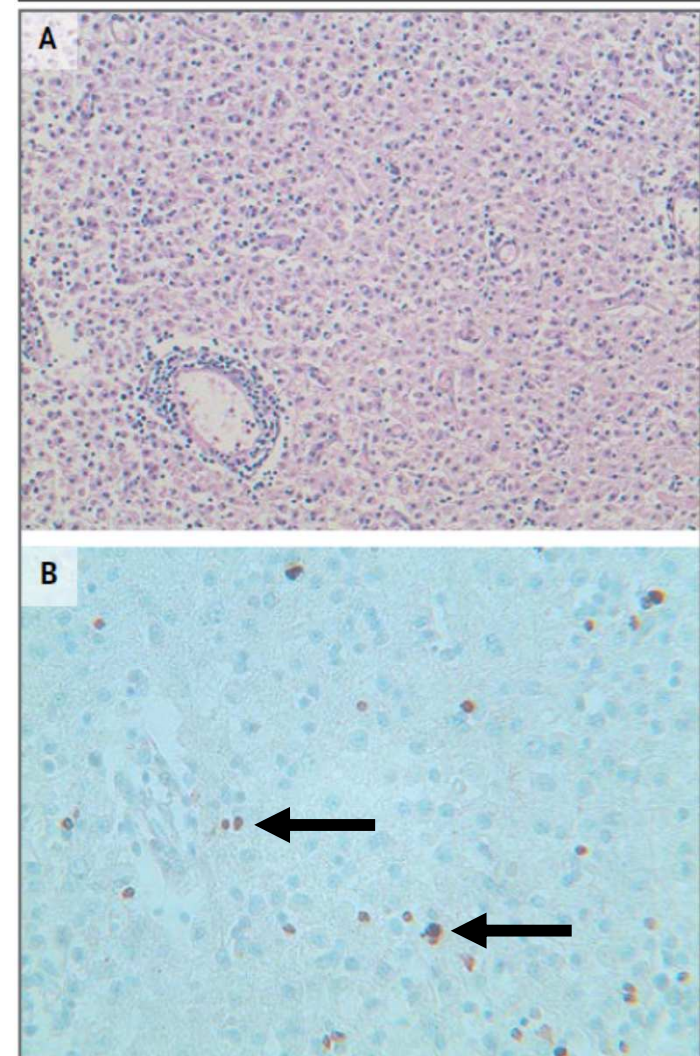


Figure 2. Findings in the Brain-Biopsy Specimen.

Panel A shows diffuse infiltration by foamy macrophages, with patchy perivascular lymphocytic infiltration (hematoxylin and eosin). No intranuclear inclusions are evident. Panel B shows positive immunohistochemical staining (brown) for herpes simplex virus.

Intrathecal Ab synthesis and herpes simplex encephalit. with neg HSV PCR

	Patient 1	Patient 2	Patient 3
Sex, age	M, 54	F, 67	F, 31
Temperature	38.8°C	38°C	38.5°C
Neurological signs	Confusion Coma Seizures Personality change	Confusion Time and place Disorientation Speech disorder	Confusion Seizures Memory loss
Delay between first signs and hospitalisation and first Lumbar puncture	Less than 2 days	Less than 2 days	Less than 2 days but probably symptoms for about 10 days
First CSF	White blood cells: 89/mm3 (lymphocytes: 32%) Protein: 0.53 g/L Glucose: normal	White blood cells: 610/mm3 (lymphocytes: 96%) Protein: 1.24 g/L Glucose: normal	White blood cells: 190/mm3 (lymphocytes: 87%) Protein: 0.98 g/L Glucose: normal
EEG recording	Focal periodic discharges in right temporal area (day 1)	Periodic lateralised epileptiform discharges in left temporal area (Day 3)	Periodic lateralised epileptiform discharges in left temporal area (Day 1)
MRI	Signs of encephalitis (day 20)	Signs of encephalitis (Day 3)	Normal
PCR for HSV	Negative: day 1, 20	Negative: day 5, 10, 15, 22	Negative: day 1, 3, 7, 17
Specific Intrathecal synthesis	Positive on day 21 after any clinical signs onset.	Positive on day 10 after any clinical signs onset.	Positive on day 17 after any clinical signs onset.
Tibbling-Link index	1.14	8.65	1.40

Tibbling-link index : $\frac{\text{IgG HSV}_{\text{CSF}} / \text{Albumine}_{\text{CSF}}}{\text{IgG HSV}_{\text{serum}} / \text{Albumine}_{\text{serum}}}$

Intrathecal synthesis or Passive transfer ?

Are there some pitfalls/ limits with HSV PCR during Herpes simplex encephalitis ?

. Role of viral load monitoring (quantitative PCR) for prognosis ?

- . one study with a positive correlation :

Domingues R, J Clin Microbiol 1998

- . current studies : no correlation

Hjalmarsson A , J Neurol 2009

Schloss L, J Med Virol 2009

- . no other biological surrogate markers for outcome ?

. Usefulness of a PCR at the end of the treatment ?

PCR HSV positive and non response to Acyclovir Therapy ?

- False positive PCR ?
- Associated autoimmune encephalitis ?
- HSV resistance ?

Acyclovir-resistant herpes simplex encephalitis in a patient treated with anti-tumor necrosis factor- α monoclonal antibodies

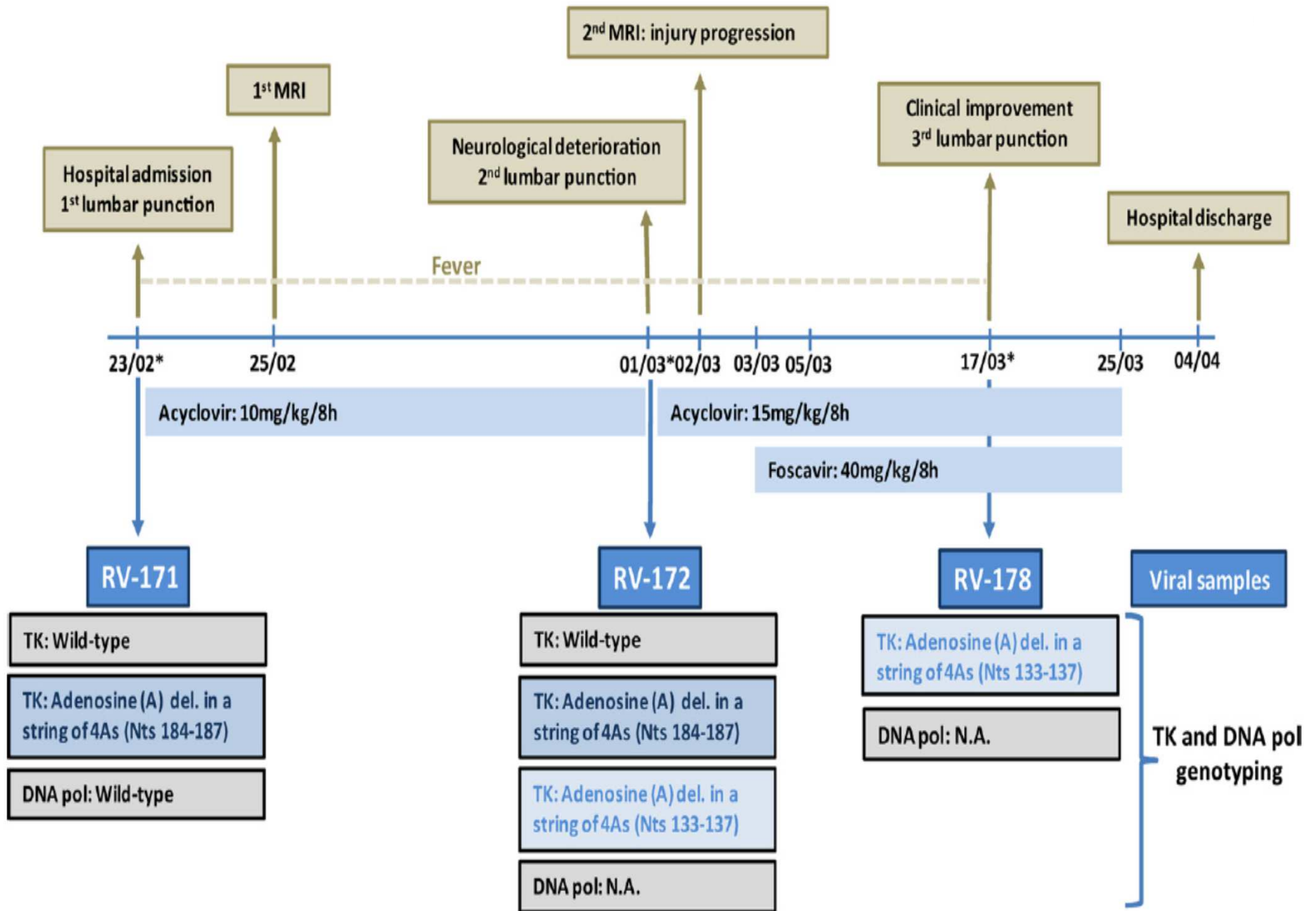
Schepers k, J Clin Virol 2014

Acyclovir Resistance in Herpes Simplex Encephalitis

Schulte E, Ann Neurol 2010

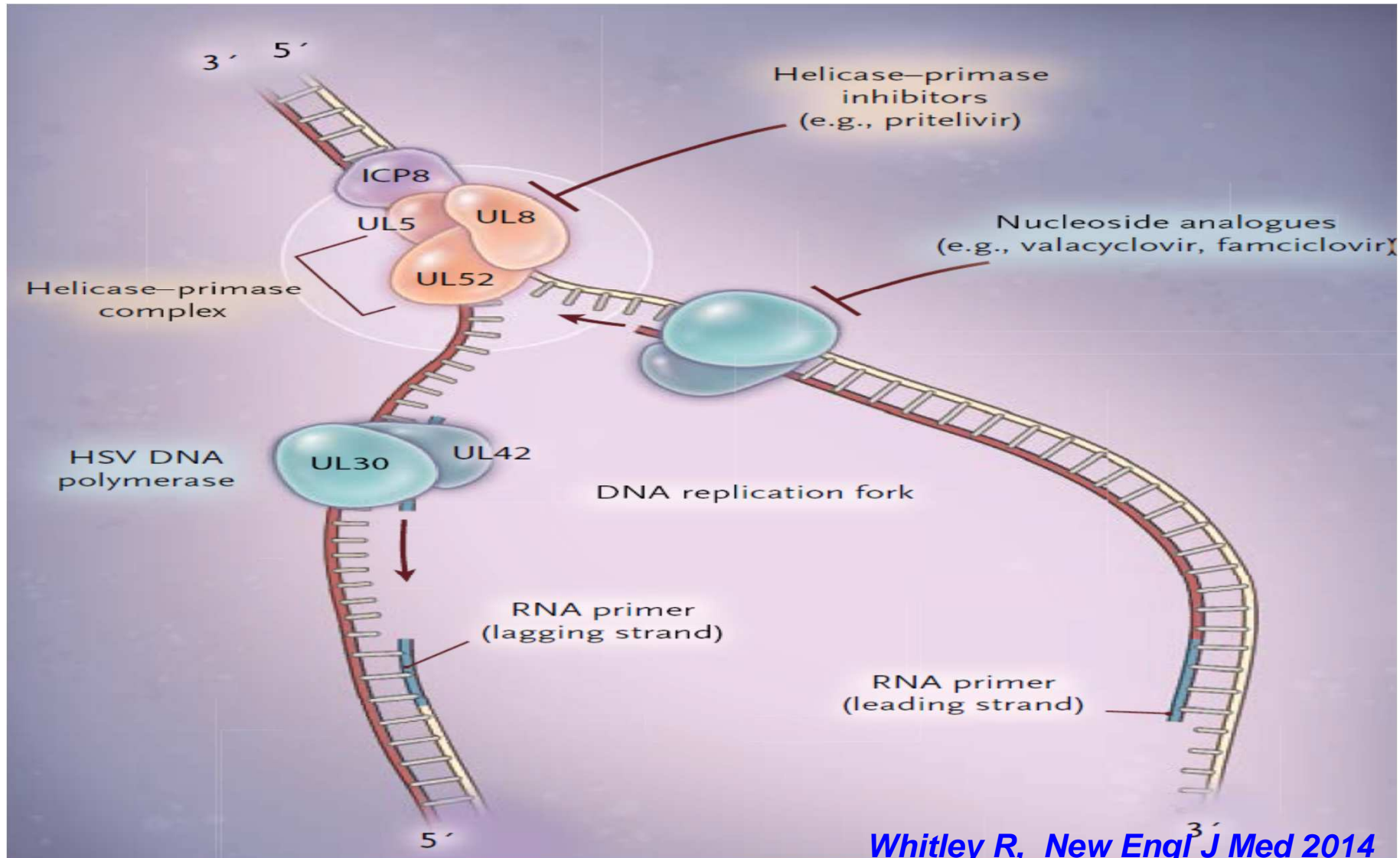
Herpes simplex virus drug-resistance: new mutations and insights

Andrei G, Curr Opin Infect Dis 2013



Helicase–Primase Inhibitor Pritelivir for HSV-2 Infection

Wald A, New Engl J Med 2014



Viral encephalitis after allogeneic stem cell transplantation: rare and different

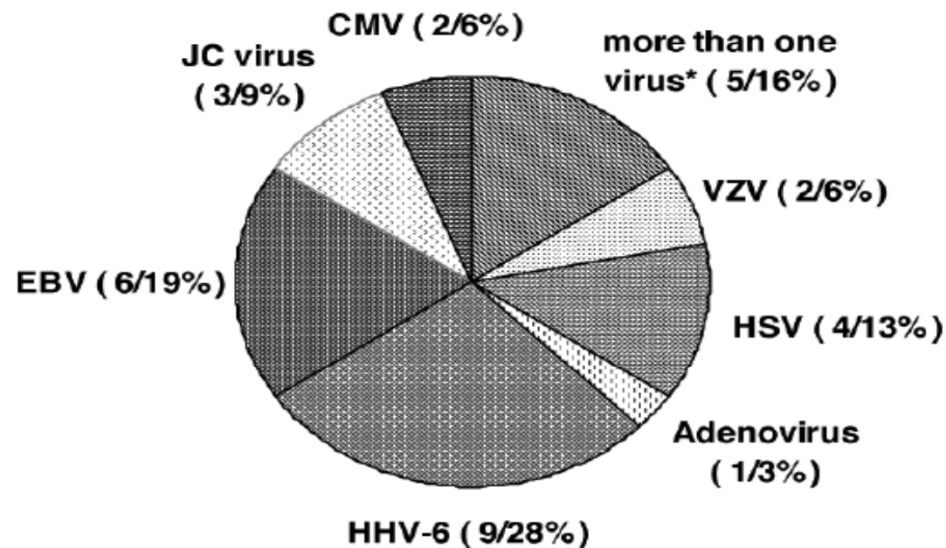
2 628 patients (1999-2009, retrospective study)

.32 patients : positive PCR for virus in CSF (1.2%) :

HHV6 > EBV > HSV

Co-infections

- . Viral encephalitis associated with T cell depletion OKT3 or alemtuzumab
- . Poor survival



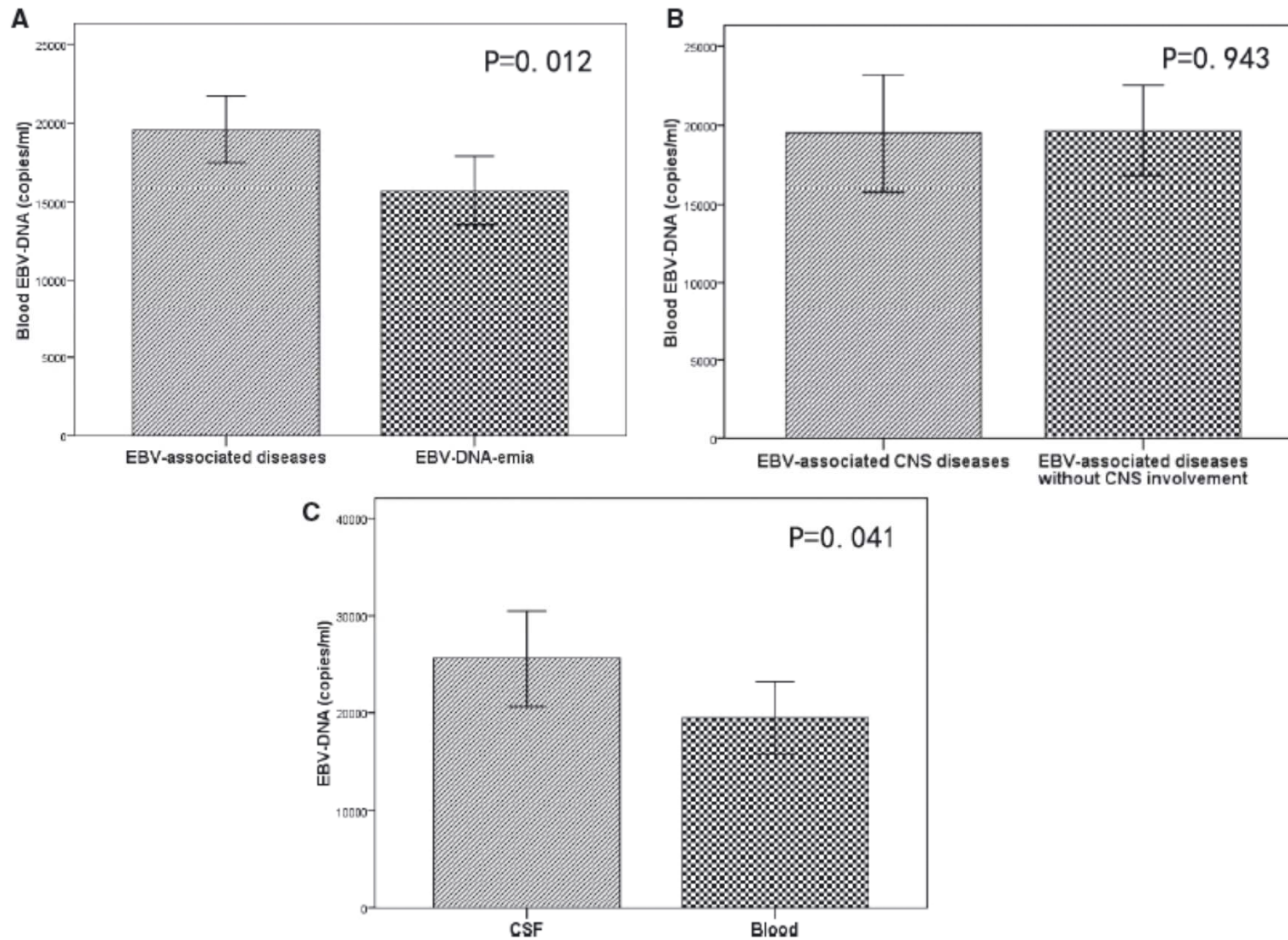
*Including CMV + HHV-6 + JC virus; HHV-6 + HHV-7; CMV + HHV-6; HSV + EBV; CMV + VZV + HSV + EBV.

Schmidt-Hieber M, Haematologica 2011

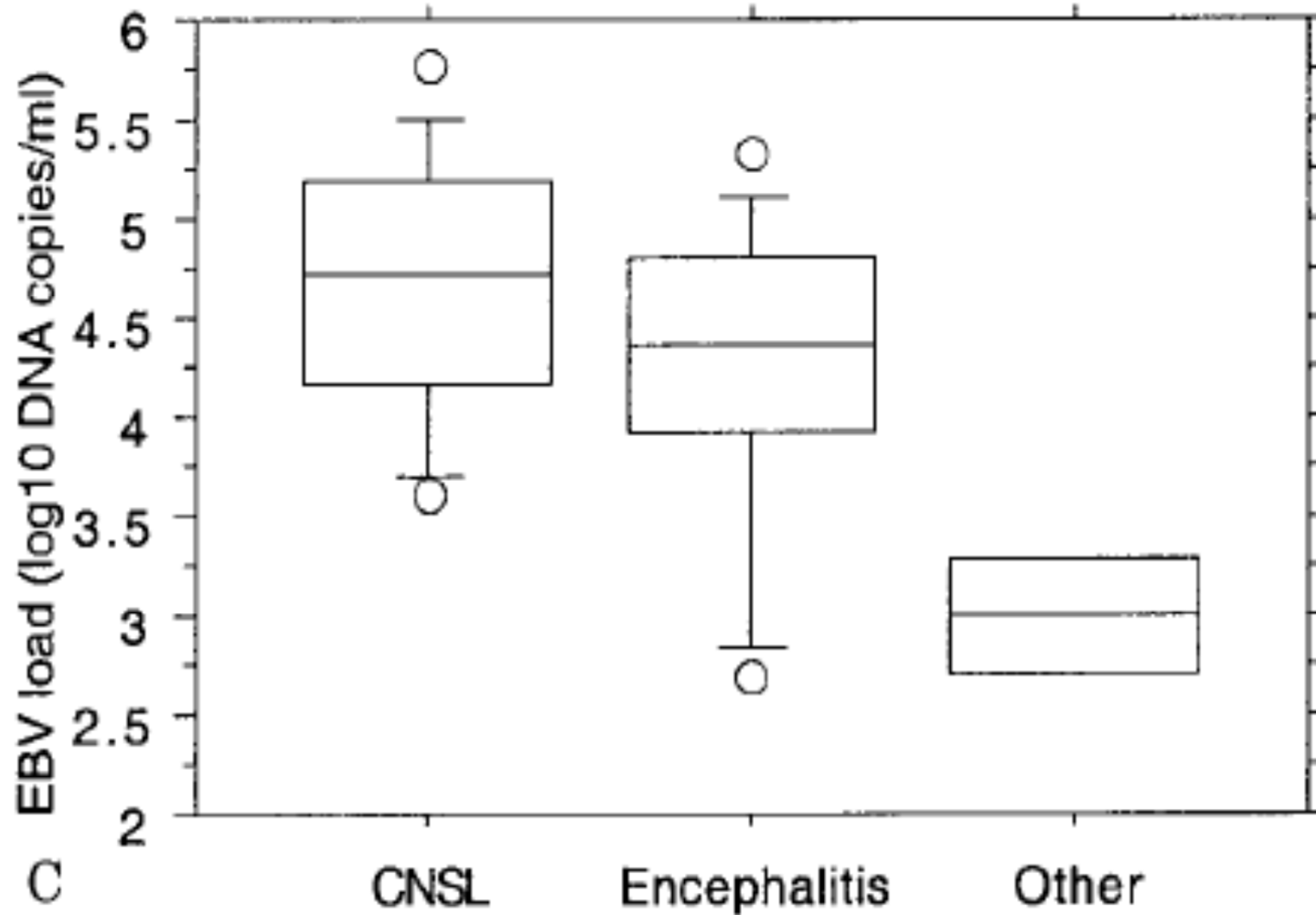
EBV encephalitis after allogeneic hemaopoietic stem cell transplantation

- .Prospective survey 172 patients (2008-2011)**
- . 3-y cumulative incidence of EBV-associated CNS diseases : 8.6 ± 2.4 %**
- . 12 cases EBV-associated CNS disease :**
 - . 8 Post Tranplant Lymphoproliferarive Disease (PTLD)**
 - 4 isolated CNS PTLD ,**
 - 4 systemic PTLD with brain involvement of whom 2 encephalitis**
 - . 3 encephalitis**
 - . 1 myelitis**

EBV load in CSF : which *threshold* ?



EBV load in CSF : which *threshold* ?



How differentiate isolated CNS PTLD from EBV encephalitis when Biopsy is impossible

.11 patients with CNS PTLD :

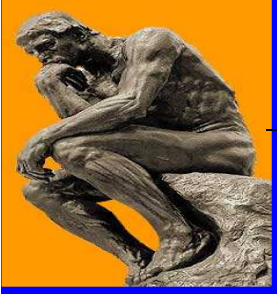
—→ 7/11 CD19 CD20 B cell

—→ 3/11 clonal rearrangement of Ig Gene

. 8 patients without PTLD :

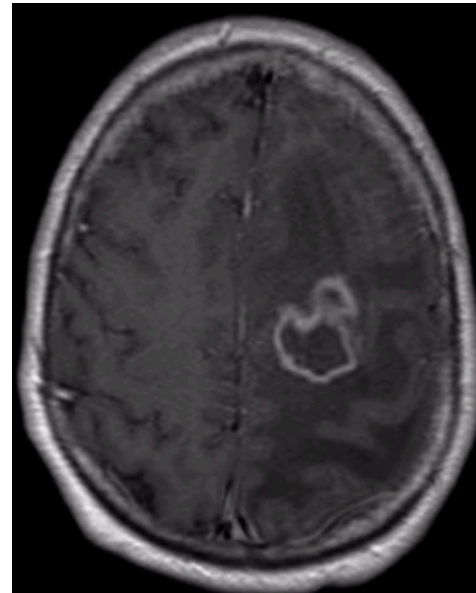
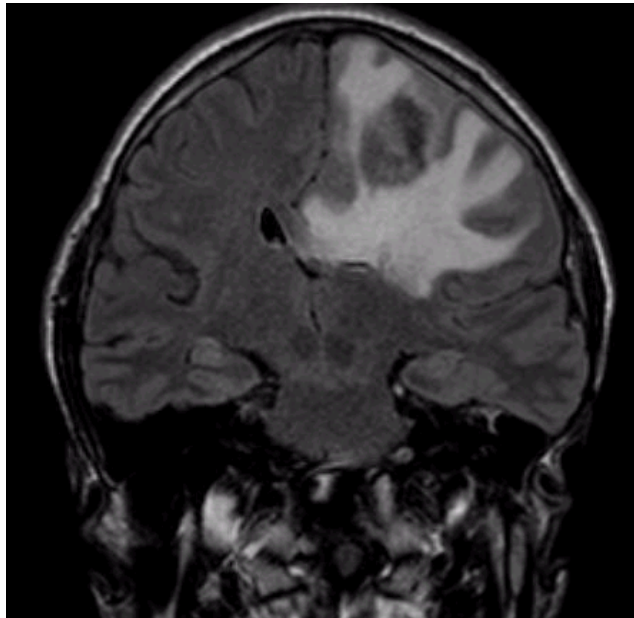
—→ no predominance of CD19 CD20 B cell

—→ no clonal rearrangement of Ig Gene



Case report

**40 year old patient renal transplantation in 2004
February 2008 Hospitalization for seizure**



Impossibility of lumbar puncture

Cerebral biopsy : inconclusive histology

EBV load in biopsy $> 2 \cdot 10^6$ copies/ ug d'ADN

Post Transplantation Lymphoproliferative Disease



Case report

- . Mr X 40 year old ,
August 2011 after Corsica holidays and notion of mosquito bites
febrile encephalitis with cerebellitis
maculo papular rash**
- . Normal CT scan and MRI TDM / IRM normale**
- . CSF : 12 cell /mm³ (80 % lymphocytes) /
negative gram stain
protein 0.56 g/L
glucose : normal**
- . Hepatic Cytolysis (Transaminases X6)**

- . Hepatitis E encephalitis :
positive IgM)
HEV PCR positive in blood / CSF / stools

- . Notion de Figatelle in Corse +++

Pig Liver Sausage as a Source of Hepatitis E Virus Transmission to Humans



Colson P J Infect Dis 2010

Figure 1. A, Figatellu purchased in a supermarket in Marseille. B, Real-time polymerase chain reaction curves for the detection of hepatitis E virus in figatellu no. 2 (tested in duplicate) and 4 negative controls. C, Packaging of a figatellu with the new recommendation to cook the sausage. *Translated from the French language as "product to cook (cook thoroughly)."

a virologist's point of view

Today and Tomorrow :
rapid and easy to use syndrome-based multiplex PCR

The Day after Tomorrow :
DNA microarrays and mass spectrometry ?
Next Generation Sequencing ?.

Serology still helpful

*always : «... recommended tests should not
supplant clinical judgment ... »*

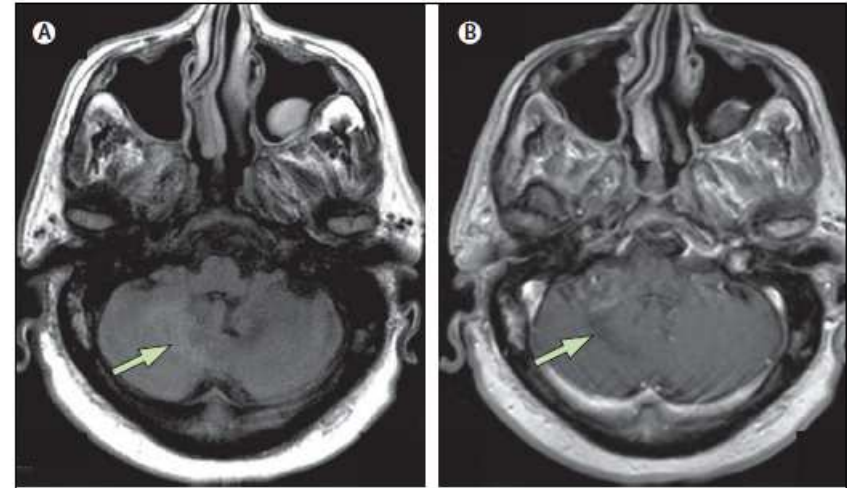


Progressive multifocal leukoencephalopathy (PML) and JC virus

Debiasy Clin Microbiol Rev 2004,

- . **CSF PCR :**

- . **Sensitivity 50- 95 %
(better on centrifuged CSF)**



- . **Specificity around 100%**

- . **High viral load in CSF (> 4.7 log units) correlated shorter survival**

- . **JCV PCR in urine or blood not predictive of PML**

Progressive multifocal leukoencephalopathy (PML) and HIV

1. HAART : Significant decrease of JC viral load :

reduced rate of positive detection

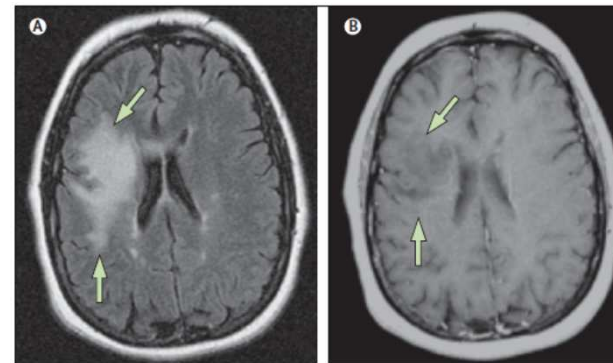
risk of false negative PCR correlated with CD4 > 100 μ l

Parameter	Results for:		P
	Pre-HAART era	HAART era	
Positive detection rate ^b	17/19 (89.5 [75.5-103.5])	23/40 (57.5 [42.1-72.9])	0.014
Specificity ^c (%)	82/83 (98.8 [96.4-101.2])	141/141 (100)	NS
Negative predictive value (%)	98	89	0.03
Positive predictive value (%)	95	100	NS

**Marzochetti A et Al ,
J Clin Microbiol ,2005**

2. Immune reconstitution inflammatory syndrome (IRIS)

up to 23% of PML in HIV patients
low or negative viral load



Tans CS et al, Lancet Neurol 2010

Progressive multifocal leukoencephalopathy (PML)

3. immunomodulatory medications (*Tans CS et al, Lancet Neurol 2010*)

- Natalizumab and MS or Crohn
- Efalizumab and psoriasis
- Rituximab

4. PCR and virus variability (*Landry MI et al , J Clin Virol 2008*)

31-year-old woman

.corticosteroid for suspicion of Multiple sclerosis
aggravation : clinical and MRI : MS /ADEM/ PLM/ Vasculitis

First CSF negative for JC PCR (VP1) : negative

Deterioration (intravenous immunoglobulin/ plama exchange)

cerebral biopsy : polyoma particules by EM

Control of the CSF with another JC PCR (large T antigen) : highly +

→ Suspicion of PLM with first PCR negative :

- Repeated test with the same or different PCR targets
- If PCR repeatedly negative consider brain biopsy

VZV encephalitis: the unknown (?) second etiology of infectious encephalitis

8 % (n=20) of the cases in the 253 French cases

- 16 PCR + in CSF :

8 adults without cutaneous involvement

7 adults with cutaneous involvement

1 boy with chicken pox

- 4 PCR negative

2 boys with acute encephalitis within varicella (1 day, 1 week)

2 adults acute encephalitis within or after zoster (3 days, 3 weeks after)

- 15 % of mortality (> 75 year-old) (vs 5% in our HSV cases)

-3 year outcome : 41 % moderate to severe sequelae (glasgow outcome scale)

VZV CNS diseases still persist but change with and varicella vaccine :

1. USA : VZV vaccine coverage (at least one dose) 90 % in 2007

2. California Encephalitis project 1998-2009 :

43 / 4021 CSF = PCR VZV positive (1%)

. 26 CSF analyzed (7 patients <16 year-old, 4 vaccinated):

.13 meningitis

.11 encephalitis (7 without Rash)

.2 ADEM (1 without rash)

decrease of VZV encephalitis in childhood

**one case (meningitis) with the Oka vaccine strain
≈10 cases of CNS disease associated with VZV
vaccine in the literature (mostly meningitis)**

Autoimmune N-methyl-D-aspartate receptor encephalitis is a differential diagnosis of infectious encephalitis

Thomas L, *J infect* 2012

Table 1 Identified etiology of the 108 patients with acute febrile encephalitis.

		N. of cases	%
Virus	HSV	17	15.7
	VZV	7	6.5
	CMV	2	1.8
	Toscana virus	1	0.9
	Influenza A	1	0.9
	Enterovirus	1	0.9
	Bacteria	<i>Mycobacterium tuberculosis</i>	5
<i>Listeria monocytogenes</i>		4	3.7
Undetermined		68	62.9
Auto-immune	NMDAR-antibody	2	1.8