Herpesviruses
Tools of diagnosis: what to use and when

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Evolution of the techniques in the virology lab
## Techniques: "Classic" methods

<table>
<thead>
<tr>
<th></th>
<th>Ag detection</th>
<th>Viral culture</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV 1-2</td>
<td>direct examination (DE)</td>
<td>+</td>
<td>+, ...</td>
</tr>
<tr>
<td>VZV</td>
<td>DE</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>CMV</td>
<td>Ag pp65 (blood)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>DE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td>NA</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>HHV6</td>
<td>NA</td>
<td>coculture</td>
<td>+</td>
</tr>
<tr>
<td>HHV7</td>
<td>NA</td>
<td>NA</td>
<td>+, but ...</td>
</tr>
<tr>
<td>HHV8</td>
<td>NA</td>
<td>NA</td>
<td>+</td>
</tr>
</tbody>
</table>
Techniques : Molecular methods

- Detection and quantification of herpesviruses
- Rapid evolution of molecular diagnosis techniques
PCR end point: detection quantification
Techniques: Molecular methods – quantification
Real time PCR (detection and quantification)

Figure 2. Model of a single amplification plot, showing terms commonly used in real-time quantitative PCR.
Rapid viral culture / Real time PCR (711 specimens)

- throat, cutaneous, genital swabs (% positive)*

<table>
<thead>
<tr>
<th></th>
<th>HSV1</th>
<th>HSV2</th>
<th>VZV</th>
<th>CMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>19</td>
<td>10.8</td>
<td>4.6</td>
<td>14</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>21.4</td>
<td>14.5</td>
<td>7.4</td>
<td>19.8</td>
</tr>
</tbody>
</table>

* most of them associated with lesions
<table>
<thead>
<tr>
<th>Virus and culture result</th>
<th>No. of specimens$^a$</th>
<th>Ct value</th>
<th>Difference between sample means (95% confidence interval)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>33.1</td>
<td>6.25</td>
<td>−2.14 (−8.39−4.12)</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>36.3</td>
<td>4.35</td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>19.5</td>
<td>4.75</td>
<td>−4.57 (−9.00−1.29)</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>24.1</td>
<td>5.95</td>
<td></td>
</tr>
<tr>
<td>HSV-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>125</td>
<td>27.0</td>
<td>6.33</td>
<td>−7.77 (−10.7−−4.79)</td>
</tr>
<tr>
<td>Negative</td>
<td>19</td>
<td>34.8</td>
<td>4.25</td>
<td></td>
</tr>
<tr>
<td>HSV-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>72</td>
<td>23.2</td>
<td>4.35</td>
<td>−10.5 (−12.6−−8.39)</td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>33.7</td>
<td>5.16</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Some specimens are missing Ct data.

$^b$ NS, not significant.
Real time PCR / nested PCR comparison in patients suspected of HSV2 meningitis

<table>
<thead>
<tr>
<th></th>
<th>N pts</th>
<th>RT</th>
<th>nested</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV2 meningitis total</td>
<td>65</td>
<td>52 (80%)</td>
<td>47 (72%)</td>
</tr>
<tr>
<td>primary</td>
<td>38</td>
<td>33 (87%)</td>
<td>33 (87%)</td>
</tr>
<tr>
<td>recurrent</td>
<td>27</td>
<td>19 (70%)</td>
<td>14 (52%)</td>
</tr>
<tr>
<td>Aseptic meningitis*</td>
<td>45</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>* 2 VZV +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(- for borrelia, TBE, HSV1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Franzen-Röhl, 2007
Prevalence of viruses detected in the CSF by PCR (1995-2001)

Real time PCR

<table>
<thead>
<tr>
<th>Virus</th>
<th>N of PCR + in CSF samples (576)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV</td>
<td>409 (71%)</td>
</tr>
<tr>
<td>HSV1</td>
<td>54 (9,4%)</td>
</tr>
<tr>
<td>CMV</td>
<td>41 (7,1%)</td>
</tr>
<tr>
<td>VZV</td>
<td>29 (5%)</td>
</tr>
<tr>
<td>HSV2</td>
<td>13 (2,3%)</td>
</tr>
<tr>
<td>JC virus</td>
<td>12 (2,1%)</td>
</tr>
<tr>
<td>EBV</td>
<td>11 (1,9%)</td>
</tr>
<tr>
<td>HHV6</td>
<td>5 (0,9%)</td>
</tr>
<tr>
<td>Parvovirus</td>
<td>2 (0,3%)</td>
</tr>
</tbody>
</table>

26% = Herpesviruses

† diagnosis by testing for different viruses
† in VZV diagnosis

Aberle, 2003
Table 1 Etiology of aseptic meningitis

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

* 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.
Table 4 Diagnostic findings for entero- and herpesviruses from CSF samples

<table>
<thead>
<tr>
<th>Virus</th>
<th>PCR</th>
<th>Culture</th>
<th>AB-1</th>
<th>AB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Aseptic meningitis, n = 144</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterovirus</td>
<td>23 (30/133)</td>
<td>14 (17/121)</td>
<td>3 (2/78)</td>
<td>0 (0/34)</td>
</tr>
<tr>
<td>HSV-1</td>
<td>2 (3*136)</td>
<td>0 (0/121)</td>
<td>0 (0/87)</td>
<td>0 (0/41)</td>
</tr>
<tr>
<td>HSV-2</td>
<td>16 (22/136)</td>
<td>0 (0/121)</td>
<td>0 (0/87)</td>
<td>2 (1/41)</td>
</tr>
<tr>
<td>VZV</td>
<td>7 (8/121)</td>
<td>0 (0/121)</td>
<td>3 (2/79)</td>
<td>3 (1/34)</td>
</tr>
<tr>
<td>Encephalitis, n = 42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterovirus</td>
<td>0 (0/35)</td>
<td>0 (0/37)</td>
<td>0 (0/13)</td>
<td>0 (0/7)</td>
</tr>
<tr>
<td>HSV-1</td>
<td>10 (4†/42)</td>
<td>0 (0/37)</td>
<td>0 (0/33)</td>
<td>9 (2†/22)</td>
</tr>
<tr>
<td>HSV-2</td>
<td>0 (0/42)</td>
<td>0 (0/37)</td>
<td>0 (0/33)</td>
<td>0 (0/22)</td>
</tr>
<tr>
<td>VZV</td>
<td>11 (4†/35)</td>
<td>0 (0/37)</td>
<td>3 (1/33)</td>
<td>9 (2†/22)</td>
</tr>
</tbody>
</table>

* In one patient, CSF PCR tests were positive for HSV-1 and VZV.
† In one patient, CSF PCR tests were positive for HSV-1 and VZV and intrathecal antibody production developed for both of these viruses.

AB-1 and AB-2 = CSF antibodies in early (1) and in follow-up (2) CSF (only intrathecal antibody production is considered); HSV = herpes simplex virus; VZV = varicella zoster 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.
Herpes simplex encephalitis:

PCR results according to days of therapy
Herpes simplex encephalitis: Intrathecal antibody production and PCR results
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
Intrathecal production of specific antibodies

1. Albumine in CSF
   ------------ has to be < 0.009
   Albumine in blood

2. specific antiviral antibodies (quant) in CSF
   Albumine in CSF
   ------------------------------------------- >1.91
   specific antibodies (quant) in blood
   Albumine in blood

= Intrathecal production of specific antibodies
CMV pp65 Antigenemia

• phosphoprotein of the viral tegument in the nucleus of PMN in blood
  = active CMV infection

N. of positive cells/100,000 cells (PMN)
± good correlation with viral load as measured by molecular methods

- neutropenia : loss of sensitivity
CMV pp65 antigenemia
Quantification of viral load by Real time PCR

Figure 2. Model of a single amplification plot, showing terms commonly used in real-time quantitative PCR.
Comparison of real-time quantitative PCR and Cobas amplicor CMV monitor.
Schaade et al, 2000
CMV viral load: pp65 Ag or real-time PCR (whole blood) in renal transplant patients
Fig. 1. Correlation between copy number of CMV DNA in whole blood and pp65-positive cells count in polymorphonuclear leukocytes. One hundred and seventeen samples from renal transplant patients of Laboratory 1 were analysed using both real-time PCR and pp65 antigenemia assay. The copy numbers of CMV DNA were plotted on a logarithmic graph against the pp65-positive cell numbers obtained by the antigenemia assay. (A) Correlation between the CMV DNA viral load obtained by the “in-house 1” PCR and the pp65-positive cell number (Spearman’s rank test $r = 0.68, p < 0.0001$). (B) Correlation between the CMV DNA viral load obtained by the CMV R-gene™ test and the pp65-positive cell number (Spearman’s rank test $r = 0.63, p < 0.0001$).
Fig. 2. Results of monitoring of CMV viral load in three individual patients after transplantation measured by Cobas Monitor, TaqMan and pp65 antigenemia. Patient 1 (monitored until discharged to another hospital) and patient 2 are liver transplant patients and patient 3 a kidney transplant recipient. The values below zero on the y axis indicate copy numbers below the detection limits of the PCR assays (Cobas <400 cps/ml and TaqMan <250 cps/ml) or negative antigenemia results. Ganciclovir treatments are marked with arrows.
Ocular Herpetic diseases

Acute retinal necrosis (ARN) syndrome:
- necrotizing retinitis
- retinal arteritis
- inflammatory reaction in vitreous and anterior chamber
- VZV 66%, HSV 22%, EBV 17% (+ VZ)
- diagnosis: PCR and intraocular antibodies
Ocular Herpetic diseases

ARN due to HSV2 (11 cases):
  mean 22.6 years (25 days – 56)
  30% bilateral involvement
  immunocompetent individuals
  associated with herpes neonate (1)
  corticoïds (3)
  trauma (1)
  chorioretinal scars (3)
  previous episodes of ARN (3)
Ocular Herpetic diseases

Complications: cataract, epiretinal membrane, retinal detachment, optic nerve atrophy

frequence: 1 cas / $2.10^6$ / an
Ocular Herpetic diseases

Diagnosis

• PCR on aqueous and/or vitreous humor
• PCR could help monitoring the treatment
• Japan : ARN due to HSV2 frequent : no preexistence of HSV1 antibodies ?
Ocular Herpetic diseases

• Necrotizing retinitis

Diagnosis: Goldmann – Witmer coefficient

specific antibodies (eye)

IgG (eye)

> 3 or 4

specific antibodies (serum)

IgG (serum)
Herpesviruses and serology

IgG and IgM (commercial kit):

- enzyme immuno assay
- chemiluminescence assay
- immunofluorescence assay
- immunoblot assay (confirmation)
Indications:

**Diagnosis of acute infection**: EBV, CMV, HHV6
- presence of IgM
- seroconversion of IgG (2 sérums)
  - negative → positive
  - "significant" increase in IgG
"Immunity" or past contact with the virus
- presence of IgG
  - ex: before organ transplantation (EBV, CMV, VZV)

**Indirect diagnosis**: HHV8
- Kaposi's sarcoma
Frequent cross-reactivity of EBV IgM and CMV IgM in serologic assays
- conventional or recombinant Ag

- short glycine-rich motifs in pUL44 and pUL57 CMV (major antigenic domain for IgM Ab during CMV)

Primary EBV induces IgM antibodies that bind to widely used diagnostic antigens in CMV IgM tests
IgG avidity

- IgG high avidity: past infection
- IgG low avidity: recent and sometimes past infection
Avidité en fonction du délai
Indice d'avidité d'infections à CMV anciennes.

Graphique montrant les résultats de l'indice d'avidité pour différentes séries de numéros de sérum. Les valeurs varient entre 0 et 1. Les points sont répartis uniformément sur l'axe des abscisses (numéro du sérum) et l'axe des ordonnées (indice d'avidité).