Divergent trends in HIV RNA levels in the cerebrospinal fluid of children and adolescents with central nervous system complications

Duiculescu D\textsuperscript{1}, Ene L\textsuperscript{1}, Tardei G\textsuperscript{1}, Achim C.L\textsuperscript{2}

\textsuperscript{1}'Dr. Victor Babes' Hospital for Infectious and Tropical Diseases, AIDS Department, Bucharest, Romania,

\textsuperscript{2}HIV Neurobehavioral Research Program, University of California at San Diego, La Jolla, CA, USA

Presented at the 3\textsuperscript{rd} HIV Pediatrics Workshop, 15 - 16 July 2011, Rome, Italy
Presented at the 3rd HIV Pediatrics Workshop, 15 - 16 July 2011, Rome, Italy

Microglia
T cell
Astrocyte
Perivascular macrophage
Monocyte
Microvascular endothelial cell

Blood
BBB
CSF

Inflammatory markers: TNFα, IL6, IL1, IFNγ, nitric oxide, Chemokines eg CCL2, CCL7

Productive infection
HIV proteins: Tat, Nef, Vpr

Latent infection
HIV-1 virion
New “bad guy”
Background:

- The extent of viral replication corresponds to HIV-1 related central nervous system (CNS) involvement \(^1\) \(^2\)
- In adults neurocognitive impairment is associated with higher cerebrospinal fluid (CSF) HIV-1 viral load (VL) \(^3\) \(^4\)
- Controlling HIV replication in the CSF in response to cART doesn’t seem to be sufficient due to persistence of immune activation and low level HIV replication in the brain tissue \(^5\)
- There are few data on CSF HIV-1 RNA levels in children especially related to HIV encephalopathy \(^6\) \(^7\)
- Since prevalence of neurological opportunistic infections in the pediatric population is lower than in adults, the significance of HIV RNA levels in the CSF was not studied

\(^1\) Di Stefano AIDS: May 1998, 12(7): 737-743
\(^2\) Eggers AIDS 2003, 17(13): 1897-1906
\(^3\) Gisslen JAIDS 1998,17 (4): 291-295
\(^4\) Ellis RJ Neurology 2000;54:927-36
\(^5\) Abdulle S et al, AIDS. 2002 Nov 8;16(16):2145-9
\(^6\) Epstein Ann Neurol. 1987 Apr;21(4):397-401
\(^7\) Patel K (2009) AIDS 23(14):1893-901
Romanian cohort characteristics

- Infected with HIV-1 subtype F in the first years of life during a short time span: 1987-1990
- Parenteral route of HIV transmission
- Currently aged 20-22 years
- Sex ratio male/female=54/46
- Common genetic background
- HAART starting 1998
- Evolution similar to adults with higher prevalence of OI
Objectives

– to compare cerebrospinal fluid (CSF) and plasma HIV RNA levels (VL) in children and adolescents with and without neurological complications

– to establish the relevance of CSF HIV RNA viral load as marker for CNS-related neurological complications
Methods:

• Cross-sectional study of HIV-1 viral load (VL), on paired plasma and cerebrospinal fluid (CSF) samples in 132 children, adolescents and young adults with parenteral-acquired HIV-1 infection, diagnosed with different neurological diseases between 2005-2011

• HIV-1 RNA load was assessed using PCR:
  – Cobas Amplicor HIV-1 Monitor, v2.5 (Roche) with detection limit of 400 copies/ml,
  – LCX (Abbott) detection limit: 178 =2.25 log10 copies/ml
  – TaqMan (Roche) detection limit: 47 (1.67 log) c/ml
  – Cobas Amplicor HIV-1 Monitor, v1.5 (Roche) detection limit” 50 (1.7 log) c/ml

• The integrity of blood-brain barrier (BBB) was estimated using the albumin index (CSF albumin/serum albumin x1000; normal value <9) starting 2009
## General characteristics of patients

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No of patients</strong></td>
<td>132</td>
</tr>
<tr>
<td><strong>Age at the moment of neurological complication (years)</strong></td>
<td>Mean: 18.7 ± 5.4; Range: 0.9-23</td>
</tr>
<tr>
<td><strong>Male/female ratio</strong></td>
<td>69/63</td>
</tr>
<tr>
<td><strong>HIV-1 transmission route</strong></td>
<td><strong>Parenteral</strong>: 116; <strong>Vertical</strong>: 6; <strong>Sexual</strong>: 10</td>
</tr>
<tr>
<td><strong>Median time from HIV-1 diagnosis to neurologic complications</strong></td>
<td>5.5 years (range 0-17)</td>
</tr>
<tr>
<td><strong>Patients with neurologic complications as first sign of HIV infection</strong></td>
<td>18 (2 with aseptic meningitis)</td>
</tr>
<tr>
<td><strong>CDC HIV class prior to neurological diagnosis</strong></td>
<td>Clinical: <strong>A</strong>: 7 patients; <strong>B</strong>: 81 patients; <strong>C</strong>: 44 patients</td>
</tr>
<tr>
<td></td>
<td><strong>Imunological (n=73)</strong></td>
</tr>
</tbody>
</table>
Overall CSF HIV loads were lower compared to plasma HIV VL's.

<table>
<thead>
<tr>
<th></th>
<th>CSF VL</th>
<th>Plasma VL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Lowest value</td>
<td>1.60</td>
<td>1.60</td>
</tr>
<tr>
<td>Highest value</td>
<td>6.59</td>
<td>6.61</td>
</tr>
<tr>
<td>Median</td>
<td>2.61</td>
<td>4.23</td>
</tr>
<tr>
<td>95% CI for the median</td>
<td>2.60 to 3.18</td>
<td>3.47 to 4.82</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>2.10 to 4.29</td>
<td>2.60 to 5.37</td>
</tr>
</tbody>
</table>
CSF HIV RNA showed a positive correlation with plasma HIV RNA.

\[ \text{Rho} = 0.68 \; P < 0.0001; \]
\[ 95\% \; \text{CI rho} \; 0.57 \; \text{to} \; 0.76 \]

CSF VL showed a positive correlation with albumin levels (rho=0.22, p=0.05) and pleocytosis (rho=0.51, p<0.001).
Naive patients had higher HIV RNA load in the CSF

Comparison between HIV RNA in CSF in naive vs treated patients

<table>
<thead>
<tr>
<th>CSF evaluation</th>
<th>Naive pts N=39</th>
<th>Pts. Previously exposed to cART but without treatment at the time of evaluation n=20</th>
<th>Pts. on cART at the time of evaluation with virological failure N=34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean HIV RNA</td>
<td>4.28</td>
<td>4.01</td>
<td>2.95</td>
</tr>
<tr>
<td>95% CI for mean</td>
<td>3.85-4.72</td>
<td>3.42-4.63</td>
<td>2.53-3.37</td>
</tr>
</tbody>
</table>

Presented at the 3rd HIV Pediatrics Workshop, 15 - 16, July 2011, Rome, Italy
Neurological conditions

Abreviations: HIVE=HIV encephalopathy, PML=progressive multifocal leukoencephalopathy, CNM= Cryptococcal meningitis, SMME= subacute myoclonic measles encephalitis

Presented at the 3rd HIV Pediatrics Workshop, 15 - 16 July 2011, Rome, Italy
### HIV RNA levels in CSF and plasma in patients with various neurological conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>VL CSF (log10c/ml)</th>
<th>VL plasma (log10c/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS defining neurological diseases (n=72)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>■ HIV encephalopathy (n=32)</td>
<td>4.62</td>
<td>4.61</td>
<td>P=ns</td>
</tr>
<tr>
<td>■ Progressive multifocal leucoencephalopathy (n=21)</td>
<td>3.13</td>
<td>4.68</td>
<td>P&lt;0.001, T=4.68</td>
</tr>
<tr>
<td>■ Inflammatory immune reconstitution syndrome (n=4, 1 PML, 1Toxo, 1CNM, 1 MTB)</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>■ Subacute myoclonic measles encephalitis (n=13)</td>
<td>2.46</td>
<td>3.78</td>
<td>P=0.006</td>
</tr>
<tr>
<td>No neurological disease N-</td>
<td>2.55</td>
<td>3.23</td>
<td>P=0.0001</td>
</tr>
</tbody>
</table>

Presented at the 3rd HIV Pediatrics Workshop, 15 - 16 July 2011, Rome, Italy
Patients with HIVE had the highest CSF HIV RNA load.

CSF HIV RNA in patients with various neurological conditions:

- HIV RNA log10 copies/ml
- HIVE
- CNM
- PML
- SMME
- non-neuro

Presented at the 3rd HIV Pediatrics Workshop, 15 - 16 July 2011, Rome, Italy
Presented at the 3rd HIV Pediatrics Workshop, 15 - 16 July 2011, Rome, Italy

- 16 of 32 pts diagnosed with HIV encephalopathy had higher CSF levels
- 3 of 11 patients with HIVE had altered BBB
• 4 of 8 patients with Cryptococcal meningitis had higher HIV loads in CSF than in plasma
• 3 of 6 patients with CNM had altered BBB

Presented at the 3rd HIV Pediatrics Workshop, 15 - 16 July 2011, Rome, Italy
Patients diagnosed with PML had lower CSF HIV loads compared to plasma.

Sample size: 22

- Lowest value: 1.60
- Highest value: 5.46
- Median: 2.91
- 95% CI for the median: 2.25 to 3.71
- Interquartile range: 2.25 to 3.72

- Lowest value: 1.60
- Highest value: 6.37
- Median: 5.00
- 95% CI for the median: 3.57 to 5.78
- Interquartile range: 3.52 to 5.78
Patients with SMME had significantly lower HIV CSF loads, possibly due to inhibitory effect of MV on HIV replication

8 of 13 pts with SMME had undetectable CSF HIV VL’s despite high plasma HIV RNA
Discussion

- Overall HIV RNA levels tend to reflect plasma levels:
  - are lower in CSF than in plasma
  - are positively correlated with plasma HIV loads, with CSF albumin levels and pleocytosis
  - similar to plasma naive patients have higher CSF VL than patients failing there ART’s
- Divergent trends in HIV RNA levels in the CSF fluid of children and adolescents were found in two circumstances
  - Patients with HIVE and Cryptococcal meningitis
    • had CSF HIV RNA loads similar to plasma
    • had altered BBB
  - Patients with PML and subacute myoclonic measles encephalitis had lower HIV RNA
    • Less inflammation?
    • Possible interaction between the 2 viruses → the inhibitory effect of measles on HIV was previously demonstrated in plasma\(^1\)\(^2\)\(^3\)

\(^1\)Moss W J et al, The Journal of Infectious Diseases 2002;185:1035-1042
\(^2\)Ruel TD et al, CROI 2007, abstract 707
\(^3\)Duiculescu D et al, IAS 2007 poster WEPDB03

Presented at the 3rd HIV Pediatrics Workshop, 15 - 16 July 2011, Rome, Italy
Limitations

• "Real life" evaluation
  – HIV RNA values were determined with different assays – ideally the threshold <20 copies/ml or 1-2 copies/ml¹
  – BBB was evaluated only since 2009
  – No genotyping studies available to demonstrate compartmentalization of HIV in CNS
• Neurocognitive impairment is associated beside HIV RNA with other factors like immune activation², and comorbidities (e.g. HCV, drug abuse)
• CSF ≠ brain
• Comprehensive quantification of HIV-1 load in brain parenchyma should utilize³:
  – proviral DNA
  – unspliced viral RNA: indicates persistence with incomplete or dysfunctional replication
  – multispliced viral RNA indicating viral replication

¹ Letendre et al. 16th CROI 2009 Abstr 484b
Practical points - HIV RNA in the CSF

- **Diagnosis**
  - for HIVE (if VL’ in CSF are similar or higher than plasma)
  - IRIS neurological OI shortly after initiation of cART, ND HIV VL’s
  - Low/undetectable VL in plasma – high VL in CSF – neurocognitive impairment → compartmentalization of HIV in the brain (! Different resistance profiles)
- **Indirect marker for other neurological opportunistic diseases** (low VL’s in patients with PML and SMME)
- **To monitor response to ART** – longitudinal measurements of VL