Latent toxoplasmosis is associated with neurocognitive impairment in a cohort of young adults with chronic HIV infection from Romania

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"Toxoplasma is a kind of marvel pathogen. It can infect everything which is warm blooded and it can be as silent as non existent in a system".
A Ubiquitous Pathogen That Keeps Its Host Healthy

The single-celled pathogen *Toxoplasma gondii* can enter the most protected parts of its host body while remaining largely undetected. In most cases it lives as a harmless tenant, but in fetuses or in people with compromised immune systems it can cause severe damage.

**Host to host**

While *Toxoplasma* can infect humans, other mammals, and birds, its relationship with cats is unique. Only in cats can the pathogen reproduce sexually to create egg-like cells.

**Cell to cell**

1. **Rapid spread**
   Within hours of infection, *Toxoplasma* can move to widely separated parts of the body. It does this by entering and controlling dendritic immune cells in the intestine.

2. **Crossing protected barriers**
   After *Toxoplasma* takes control of a dendritic cell, it can use the cell as a Trojan horse to cross protected barriers. In this way it can reach defended organs like the brain.

3. **Entering a cell**
   *Toxoplasma* can infect almost every type of cell. It enters by pushing against the membrane and pulling it over itself. The cell seals behind, leaving the pathogen in a protective bubble.

**Sources:** Antonio Barragan, Karolinska Institute and Swedish Institute for Infectious Disease Control; J. F. Dubey, Journal of the American Veterinary Medical Association
High quantities of dopamine released by *T. gondii* may be responsible for clinical behavioural changes (*PLoS One* 2011, 6:e23866)

The presence of dopamine induces increased production of tachyzoites and destruction of the cysts walls (*J Parasitol* 2012, 98:1296-1299)

Toxoplasma upregulated the miR-132 and is associated with changes in dopamine receptor signalling (*Neuroscience*. 2014 May 30;268:128-38)

During its life cycle, Toxoplasma interacts with about 3000 genes and proteins, including susceptibility genes for Alzheimer disease, Schizophrenia, and mood disorders (*J Pathog.* 2013, 965046)
Latent Toxoplasma infection was linked to psychiatric conditions & behavioral changes

- **The additional diagnosis of a personality disorder in psychiatric patients** Hinze-Selch, D., et al. (2010). Folia Parasitol (Praha) 57(2): 129-135
Latent toxoplasmosisis and cognition


- **Affecting cognitive function in certain groups** (Gale, S. D., et al. (2014). Parasitology: 1-9) → significant interactions between latent toxoplasmosisis and
  - the poverty-to-income ratio
  - educational attainment
  - race-ethnicity
Latent Toxoplasmosis – brain disturbances in HIV-infected subjects

- No significant association between positive Toxoplasma serology and psychiatric disorders (El Lakkis et. al, JAIDS (2015) 68(1) p e8-e9)
  - high baseline prevalence of psychiatric disorders
  - data collected from electronic medical records

- Older subjects with latent toxoplasmosis trended towards worse neurocognitive functioning and higher anti-Toxoplasma IgG titers were associated with worse functioning (Bharti A, et al, In: 19th International AIDS Conference. Washington DC, USA; 2012)
Objective

- We aimed to evaluate the possible contribution of latent infection with *T. gondii* on
  - neurocognitive performance
  - depression
  - suicidal risk
  - disturbances associated with frontal-subcortical circuitry damage
  - risk taking behaviours

in a group of young adults with chronic HIV infection since childhood
Methods

Study participants
- 194 HIV+ participants in the Romanian HIV Pediatric cohort who were infected with HIV in their first years of life (early 1990s) by parenteral non-IDU route
- 51 HIV- age matched participants

Neurocognitive assessment
- Standardised battery of tests assessing seven cognitive domains
- Neurocognitive impairment (NCI) was estimated using the global deficit score (GDS) with a cut-off > 0.5
- Individual test deficit scores, determined via demographically-adjusted T scores generated from a healthy population of Romanian young adults, ranged from 0 (T score of > 40) to 5 (T score < 20)
Depression, psychiatric disorders and risk taking behaviors

- The Beck II depression inventory
  - 0–13 = minimal symptoms
  - 14–19 = mild depression
  - 20–28 = moderate depression
  - 29–63 = severe depression

- The Frontal System Behaviour Scale (FrSBe)
  - 3 sub-scales: apathy, disinhibition, and executive dysfunction.

- The Risk Assessment Battery (RAB) is a self-administered, multiple choice questionnaire, assessing needle sharing practices and sexual activity associated with HIV transmission.

- MINI-International Neuropsychiatric Interview (MINI-Plus), evaluated DSM-IV criteria for current/past major depression and current/past suicidal risk
## Characteristics of the participants

<table>
<thead>
<tr>
<th>Characteristics of the participants</th>
<th>HIV-(n = 51)</th>
<th>HIV+(n = 194)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n; %)</td>
<td>28; 54.9%</td>
<td>94; 48.4%</td>
</tr>
<tr>
<td>Age (mean; SD)</td>
<td>24.2(2.4)</td>
<td>24.0 (1.5)</td>
</tr>
<tr>
<td>Education**</td>
<td>13.3 (2.6)</td>
<td>12.1 (2.8)</td>
</tr>
</tbody>
</table>

### HIV characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HIV-</th>
<th>HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time since estimated HIV transmission (years)¹</td>
<td>-</td>
<td>23.7; 22.8-24.4</td>
</tr>
<tr>
<td>Time since HIV-diagnosis (years)¹</td>
<td>-</td>
<td>14.8; 9.68-17.54</td>
</tr>
<tr>
<td>CD4 Current T-cells/µL¹</td>
<td>-</td>
<td><strong>479</strong>; 273-713</td>
</tr>
<tr>
<td>CD4 nadir cells/µL¹</td>
<td>-</td>
<td><strong>93</strong>; 22-190</td>
</tr>
<tr>
<td>Time since CD4 nadir years¹</td>
<td>-</td>
<td>6.63; 1.57-11.46</td>
</tr>
<tr>
<td>AIDS defining diseases (n; %)</td>
<td>-</td>
<td>100 (51.5%)</td>
</tr>
<tr>
<td>HIV RNA in plasma undetectable (n; %)</td>
<td>-</td>
<td>118; 58.8%</td>
</tr>
</tbody>
</table>

### ART characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HIV-</th>
<th>HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Currently taking cART (n; %)</td>
<td>-</td>
<td>178 (91.7%)</td>
</tr>
<tr>
<td></td>
<td>HIV-(n = 51)</td>
<td>HIV+(n = 194)</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td><strong>Toxoplasmosis characteristic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG Toxo positive (n; %)</td>
<td>18; 35.3%</td>
<td>63; 32.4%</td>
</tr>
<tr>
<td>IgG Toxo IU/ml among positive participants</td>
<td>937; 232-1291</td>
<td>1090; 482-1604</td>
</tr>
<tr>
<td><strong>Neurobehavioral characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDS impaired (n, %)</td>
<td>6;11.7%</td>
<td>71; 36.5%</td>
</tr>
<tr>
<td><strong>Depression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDI-II depression score &gt;13 (n; %)*</td>
<td>1; 1.9%</td>
<td>23; 11.8%</td>
</tr>
<tr>
<td>Major depression diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>current</td>
<td>1; 1.96%</td>
<td>6; 3.09%</td>
</tr>
<tr>
<td>past</td>
<td>7; 13.72%</td>
<td>29; 14.94%</td>
</tr>
<tr>
<td><strong>FrSBe (n, %)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apathy raw*</td>
<td>24.0; 6.1</td>
<td>26.6; 7.4</td>
</tr>
<tr>
<td>Disinhibition Raw Score</td>
<td>24.3; 6.6</td>
<td>25.4; 7.5</td>
</tr>
<tr>
<td>Executive dysfunction*</td>
<td>30.2; 8.2</td>
<td>33.2; 8.5</td>
</tr>
<tr>
<td>Total raw*</td>
<td>44.5; 13.2</td>
<td>48.5; 13.7</td>
</tr>
<tr>
<td><strong>Suicidal risk (n; %)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current *</td>
<td>0; 0.0%</td>
<td>14; 7.2%</td>
</tr>
<tr>
<td>Past *</td>
<td>1; 1.9%</td>
<td>23; 11.8%</td>
</tr>
</tbody>
</table>
Latent Toxoplasma infection may result in increased cognitive difficulties in co-infected individuals.

Anti-Toxoplasma antibodies were associated with a 60% increased relative risk of NCI ($\chi^2 = 6.3$, RR = 1.6, $P=0.001$).
Multivariable models examining cognitive performance (GDS and T scores) using 2-way ANOVAS and logistic regression

Controlling for HIV

<table>
<thead>
<tr>
<th></th>
<th>HIV-/Toxo-</th>
<th>HIV-/Toxo+</th>
<th>HIV+/Toxo-</th>
<th>HIV+/Toxo+</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDS (sqrt)</td>
<td>0.20 (.21)</td>
<td>0.29 (31)</td>
<td>0.44 (.44)</td>
<td>0.59 (.48)</td>
<td>HIV***, Toxo*</td>
</tr>
<tr>
<td>Mean</td>
<td>50.5 (5.1)</td>
<td>48.7 (6.4)</td>
<td>47.3 (6.1)</td>
<td>44.8 (5.5)</td>
<td>HIV***, Toxo**</td>
</tr>
<tr>
<td>Executive</td>
<td>50.8 (6.5)</td>
<td>50.0 (7.1)</td>
<td>47.4 (7.2)</td>
<td>45.7 (7.6)</td>
<td>HIV**</td>
</tr>
<tr>
<td>Verbal</td>
<td>50.1 (8.5)</td>
<td>47.8 (7.3)</td>
<td>50.3 (7.5)</td>
<td>47.7 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Working Memory</td>
<td>50.0 (7.5)</td>
<td>50.3 (11.2)</td>
<td>45.9 (9.6)</td>
<td>44.3 (9.2)</td>
<td>HIV**</td>
</tr>
<tr>
<td>Learning</td>
<td>50.7 (81)</td>
<td>49.0 (9.7)</td>
<td>46.3 (9.1)</td>
<td>43.2 (8.1)</td>
<td>HIV***, Toxo*</td>
</tr>
<tr>
<td>Memory</td>
<td>50.5 (7.5)</td>
<td>49.4 (10.5)</td>
<td>46.4 (9.8)</td>
<td>42.4 (10.2)</td>
<td>HIV***, Toxo**</td>
</tr>
<tr>
<td>Motor</td>
<td>49.5 (9.1)</td>
<td>48.8 (7.7)</td>
<td>46.4 (9.5)</td>
<td>44.3 (10.6)</td>
<td>HIV*</td>
</tr>
<tr>
<td>SIP</td>
<td>50.9 (6.3)</td>
<td>47.2 (7.4)</td>
<td>47.2 (7.9)</td>
<td>44.2 (7.0)</td>
<td>HIV**, Toxo **</td>
</tr>
</tbody>
</table>

* p < .05 , ** p < .01 , *** p < .001
Effects of toxoplasma on NCI within the HIV+ group with undetectable HIV load (n=118)

* * * *

*p<0.05
### Relationship between Toxo status and risk behaviors

<table>
<thead>
<tr>
<th>FrSBe (raw score)</th>
<th>HIV-/Toxo-</th>
<th>HIV-/Toxo+</th>
<th>HIV+/Toxo-</th>
<th>HIV+/Toxo+</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apathy</td>
<td>24.1 (6.2)</td>
<td>23.8 (6.3)</td>
<td>26.8 (7.1)</td>
<td>26.1 (8.1)</td>
<td>0.14</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>24.4 (6.7)</td>
<td>24.1 (6.6)</td>
<td>26.1 (7.6)</td>
<td>23.9 (7.1)</td>
<td>0.20</td>
</tr>
<tr>
<td>Executive</td>
<td>30.8 (7.9)</td>
<td>29.1 (8.9)</td>
<td>33.6 (8.7)</td>
<td>32.3 (8.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Total</td>
<td>79.2 (18.5)</td>
<td>76.9 (20.2)</td>
<td>86.4 (20.7)</td>
<td>82.3 (20.5)</td>
<td>0.10</td>
</tr>
<tr>
<td>Drug use (% none)</td>
<td>96.7%</td>
<td>94.4%</td>
<td>96.2%</td>
<td>96.8%</td>
<td>0.87</td>
</tr>
<tr>
<td>Sex activity (median, IQR)</td>
<td>4 (2.5,5)</td>
<td>4 (2,5)</td>
<td>2 (2.4)</td>
<td>2 (0.3)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Regression models:
- HIV & Toxo: no effect
- HIV: higher apathy (p=0.023), executive (p=0.019), and total (p=0.034)
- Toxo: no effect

* HIV-ve participants had higher sexual risk-taking behaviours
No effect of latent toxoplasmosis

- current or past depression
- current or past suicidal risk

Based on MINI-Plus evaluation
Relationship between Toxo IgG levels
cognitive performance & behavioral changes

The group with lower levels of Toxo IgG had

• **higher** disinhibition (25.9 [7.8] vs. 21.7 [5.5], \( p=0.016 \))
• **higher** dysexecutive functioning (34.3 [9.0] vs. 30.1 [6.1], \( p=0.036 \))
• **higher** percentage of individuals with at least mild depression on the BDI (24.2% vs. 3.3%, \( p=.028 \)).
Discussion (1)

1. Toxo and cognitive performances
   - Detectable anti-Toxoplasma IgG antibodies were associated with a greater risk of NCI in HIV+ and HIV-participants

   **Latent Toxoplasmosis can be a potential confounder in attributing the cause of NCI to HIV**

2. Latent Toxo infection was not associated with
   - risk behaviours
   - major depression
   - suicidal thoughts
Discussion (2)

- Lower Toxo levels were associated with higher NCI rates and indicators of frontal systems dysfunction. **Toxoplasma may exert its negative effects as a result of slow and cumulative effects.**
- Further studies and a longitudinal follow-up are warranted to determine the long-term impact of latent toxoplasmosis on NCI and also in behavioural changes and psychiatric conditions of HIV-infected patients.
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