Torque Teno Virus as a new biomarker for immune-competence in HIV infected patients

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on behalf of the RESINA-Study Team
Torque Teno Virus (TTV)

- Former called Transfusion Transmitted Virus
- Single strand, negative-sense, non-coated DNA virus
- belongs to the group of anelloviruses that compose a large fraction of the human total blood virome
- Not known to cause any clinical manifestations in humans
- High prevalent in the regular population with reported infection rates of >90%
  - 69% in 551 healthy blood donors in southern Brazil (Mazzola, Saito et al. 2015)
  - 6, 34 and 90% of healthy Japanese children aged 1, 4 and 42 months, and in 84% of adults. (Naganuma, Tominaga et al. 2008)
TTV as immune marker

- proposed as a marker of immune function
  - in patients receiving immunosuppression after *solid organ transplantation* (e.g. lung, kidney, liver) (Görzer et al., 2015; Schiemann et al., 2017; Kazemi et al., 2015)
  - in patients following *allogeneic hematopoietic stem cell transplantation* (HSCT) (Gilles et al., 2017; Albert et al., 2018)
  - HIV infected patients (Shibayama et al., 2001; García-Álvarez et al., 2012)

- Helps to estimate the risk of opportunistic infections, post-transplant complications and antibody mediated organ rejection
viral loads of TTV were significantly higher in the HIV-group and the HIV/HCV-group than the Control-group (p<0.05)
viral loads of TTV were significantly higher in HIV infected and HIV/HCV-coinfected patients with HIV viral load $\geq 50$ copies/mL ($p<0.05$)
**TTV load and CD4 cell count**

Table 3. Comparison of the titres of TT virus DNA detectable by untranslated region polymerase chain reaction or N22 polymerase chain reaction in HIV-infected patients stratified by various demographic features.

<table>
<thead>
<tr>
<th>Feature</th>
<th>UTR PCR</th>
<th>N22 PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>TTV DNA (10^9/ml)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>123</td>
<td>4.6 ± 1.2*</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–39</td>
<td>83</td>
<td>4.4 ± 1.1</td>
</tr>
<tr>
<td>40–69</td>
<td>60</td>
<td>4.7 ± 1.2</td>
</tr>
<tr>
<td><strong>Co-infection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>23</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td>HBV</td>
<td>10</td>
<td>5.1 ± 1.1</td>
</tr>
<tr>
<td>(−)</td>
<td>110</td>
<td>4.5 ± 1.2</td>
</tr>
<tr>
<td><strong>Risk factor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemophilia</td>
<td>10</td>
<td>4.6 ± 1.3</td>
</tr>
<tr>
<td>Sexual contact</td>
<td>125</td>
<td>4.5 ± 1.1</td>
</tr>
<tr>
<td>Others</td>
<td>8</td>
<td>5.3 ± 1.8</td>
</tr>
<tr>
<td><strong>HIV viral load</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10^4/ml</td>
<td>67</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>≥ 10^4/ml</td>
<td>76</td>
<td>4.8 ± 1.2</td>
</tr>
<tr>
<td><strong>AIDS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>49</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>No</td>
<td>94</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td><strong>CD4 cell count</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–100/mm³</td>
<td>46</td>
<td>5.2 ± 1.5</td>
</tr>
<tr>
<td>101–943/mm³</td>
<td>97</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>total</td>
<td>143</td>
<td>4.5 ± 1.2</td>
</tr>
</tbody>
</table>

- Inverse relationship between the TTV load and the CD4 cell count in HIV infected patients

Shibayama et al., 2001
no correlation between T-cell activation and anelloviruses levels
During HIV infection immunodeficiency occurs and finally leads to AIDS.

Routine diagnostics:
- Viral load measurement
- Determination of the number of CD4+-T-cells

Viral load as a marker of the driving force of immune destruction.

CD4+-T-cell count shows the degree of destruction that occurred already.

No biomarker for the activity of the immune system, which shows the immune competence of the immune function:
- E.g. evaluation of induction-maintenance ART strategies
  - Possibility of ART simplification/reduction
- E.g. individualized concepts regarding screening for AIDS-diseases or Hodgkin’s lymphoma
Objective

- To evaluate the presence and load of TTV in peripheral blood as a new biomarker for immune-competence in HIV infected persons

Hypothesis

- Levels of plasma TTV DNA in HIV-1 infected patients
  1. Predict the degree of immune-recovery after ART initiation
  2. Correlate with risk of AIDS events after ART initiation

→ TTV load can be used as a new biomarker giving additional information for the monitoring of ART
European cooperation project: EuResist

Germany (University of Cologne and University of Düsseldorf)

Italy (University of Siena; Maurizio Zazzi, Andrea de Luca)

Schweden (Karolinska Institute; Anders Sönnerborg)

The project is divided into two sub studies:

1. **Pilot study**: To study whether TTV load is associated with immune recovery in asymptomatic HIV-infected patients with stable virological suppression under ART

2. **Case control study**: TTV viremia is associated with the risk of AIDS events given a certain level of CD4
Pilot study - Inclusion criteria

- HIV-1 infection (RESINA study)
- No AIDS-Event before start of ART or during the first 3 months on ART
- CD4 cell count < 500 / µl at the start
- Viral load decrease to < 200 HIV RNA copies per ml without rebound in the subsequent one year
Patients classification

- three groups depending on the CD4 cell increase after 1 year ART

<table>
<thead>
<tr>
<th>Group</th>
<th>CD4 increase (cells/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>B</td>
<td>50 – 200</td>
</tr>
<tr>
<td>C</td>
<td>&gt; 200</td>
</tr>
</tbody>
</table>

- three groups depending on the CD4 cell count at baseline

<table>
<thead>
<tr>
<th>Group</th>
<th>CD4 baseline (cells/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>2</td>
<td>100 - 300</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 300</td>
</tr>
</tbody>
</table>
Material

- Plasma samples from RESINA cohort, selected independent of HI viral load
- Collected from 2001 to 2016
- Baseline samples -> **before** ART initiation
- One sample per patient
**Methods**

- DNA extraction of plasma samples
- TTV-DNA quantification by use of Realtime-PCR
- Primers and probe:
  - **AMTS**
    - $5'$-GTG CCG IAG GTG AGT TTA-3'$
    - 18 bp
  - **AMTAS**
    - $5'$-AGC CCG GCC AGT CC-3'$
    - 14 bp
  - **AMTPTU**
    - $5'$-FAM-TCA AGG GGC AAT TCG GGC T-3'TAMRA
    - 19 bp
- PCR protocol and standards from Vienna
  - standards were stabilized in Cologne
- Statistical elements were performed with ANOVA

https://www.biocompare.com/Product-Reviews/167240-Solid-workhorse-machine/
301 samples were analysed for TTV plasma levels

Group A underrepresented due to low number of patients fulfilling the inclusion criteria
TTV prevalence in HIV-infected patients

- 96% TTV plasma positive
  - only 12/301 TTV negative patients (4%)
- Patients with Low CD4 gain in the first year of ART (group A) showed all TT viremia (100%)
TTV viral load correlates with CD4 cell count

- TTV plasma DNA is significantly increased with reduced CD4 cell counts ($p=0.0074$)
TT viral load correlates with CD4 values at therapy start

- Significantly higher TT viral loads with lower CD4 cell counts (p=0.0171)
TT viremia depending on CD4 gain

- Group A 100% TTV positive
- TT viremia was increased with retarded CD4 reconstitution (n.s.)
- TTV DNA <2400 cop/ml is predictive for a CD4 increase > 50 cell/µl in the first year on ART

A: CD4 gain < 50 cells/µl
B: CD4 gain 50 - 200 cells/µl
C: CD4 gain > 200 cells/µl
Summary and conclusions

- High TTV prevalence in RESINA cohort (96%)
- Significantly higher TT plasma levels with lower CD4 cell count before ART initiation
- TTV DNA <2400 cop/ml is predictive for an adequate immune reconstitution
- Multiple correlation analysis (age, sex, HIV-RNA)
  - → no impact on CD4 gain (data not shown)

Conclusion

- TTV plasma levels could help predict the degree of immune-recovery after ART initiation
Next steps:

- Case controle study (Pia Esser, Cologne)
  - TTV viremia is associated with the risk of AIDS events given a certain level of CD4

- Longitudinal TTV monitoring
  - Prospective screening of HIV patients for TTV DNA
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