Sudden viral load increase as an indicator of HIV-1 superinfection in HAART-naive HIV-infected patients

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Background

- HIV-1 superinfection, defined as infection with a second strain of HIV-1 at least one month after a primary infection and following documented seroconversion.
- Increasingly recognised since the first reports of its occurrence in humans in 2002\(^1\).
- Drives HIV genetic diversification through recombination.
- It reveals ongoing transmission risk, and may result in accelerated disease progression and acquisition of drug resistant strains\(^2\).
- Occurs in both early and established infection.
- The true incidence is the subject of much debate.
- Studies investigating its incidence in men who have sex with men (MSM) are limited.

Aim

- To determine the frequency of occurrence of superinfection events among HAART-naïve MSM who showed a sudden increase in plasma viral load during routine clinical monitoring.
Methods

- The clinic database was screened for eligible patients.
- HAART-naïve MSM who showed a viral load increase of $\geq 0.5 \log_{10} \text{cps/ml}$ relative to two previous stable consecutive measurements.
- For the initial screening, *Pol* gene sequences (RT aa 1-335; PR aa 1-99) were obtained from plasma RNA collected before (=baseline) and after (=follow-up) the viral load increase by population sequencing*.

* ViroseqTM HIV-1 Genotyping system (Celera Diagnostics, USA).
Study Population

- 138 eligible patients (from 572 patients)
- 47 had baseline and follow-up samples available for analysis.

<table>
<thead>
<tr>
<th>Age</th>
<th>Median (range)</th>
<th>37(24-57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td>White</td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td>Black Caribbean</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>Black African</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>3%</td>
</tr>
<tr>
<td>Viral load increase log$_{10}$ copies/ml</td>
<td>Median (range)</td>
<td>0.6 (0.5-2.2)</td>
</tr>
</tbody>
</table>
Phylogenetic analysis

- Sequence subtyping was carried out using RIP3.0 and REGA subtyping tools.

- Identification of genetically divergent sequence pairs was carried out using an HKY85 model in PAUP, with further characterization carried out using PhyML3.0.

- Scanning for potential recombination was carried out using SimPlot distance plotting and Recombination Detection Program v3.41.
Results

- 45/47 (96%) patients showed no evidence of sequence divergence
- 2 (4%) showed substantially divergent *pol* sequences between baseline and follow up samples
  - White patients aged 28 and 41 years
- Patient 1 had seroconverted during the previous 6 months, whereas Patient 2 had established infection
- Both patients were infected with subtype B prior to VL increase and showed evidence of the presence of a divergent subtype B following VL increase
Phylogenetic analysis of Pol population sequences

Maximum likelihood trees drawn in PhyML3.0 with pol population sequences from each patient and using 243 subtype B reference sequences from Los Alamos database.
## Resistance mutations found in pre-VL load increase and post-VL increase

### Patient 1

<table>
<thead>
<tr>
<th>Date</th>
<th>RT mutations</th>
<th>PR mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 05</td>
<td><strong>None</strong></td>
<td>None</td>
</tr>
<tr>
<td>Oct 05</td>
<td>V179D G33E</td>
<td>None</td>
</tr>
</tbody>
</table>

### Patient 2

<table>
<thead>
<tr>
<th>Date</th>
<th>RT mutations</th>
<th>PR mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 04</td>
<td><strong>M41L T215S</strong></td>
<td>L63P, I93L</td>
</tr>
<tr>
<td>Oct 05</td>
<td><strong>None</strong></td>
<td>L10V</td>
</tr>
</tbody>
</table>
Patient histories: Patient 1

Figure 1: Kinetics of CD4 count and plasma viral load in Patient 1

Presented at the 8th European HIV Drug Resistance Workshop, March 17-19 2010, Sorrento, Italy
Clonal analysis - Methods
Patient 1

- Clones were generated from plasma viral RNA at baseline (50 clones) and from CD4-derived proviral DNA after the increase (50 clones).

- The CD4 cells were separated from peripheral blood mononuclear cells (PBMC) using magnetic beads.

- PCR products (1.4kb) containing the whole protease and two thirds of RT were amplified in 10 separate reactions, pooled and cloned using TOPO TA technology.
Clonal analysis - Results
Patient 1

Maximum-likelihood trees drawn in PhyML3.0. Pre-VL increase sequences highlighted in red; post-VL increase sequences highlighted in blue. Bootstrap values showing 100% support are indicated (1000 replicates)

Post-viral load increase

Pre-viral load increase
Antibody neutralisation
Patient 1

- Antibody neutralisation activity of early and late sera was measured as a function of a reduction in Tat-induced luciferase reporter gene expression in TZM-bl cells after a single round of infection.
- No significant neutralisation was demonstrated against reference, heterologous subtype B viruses, as well as subtypes A, C and CRFO2 strains.
Patient 1: Follow-up

- The patient continued to engage in high risk sexual behaviour
- Over the subsequent six months acquired primary syphilis and primary genital herpes with documented HSV-2 antibody seroconversion
Patient histories: Patient 2

- Evidence of transmitted drug resistance (M41L, T215S), established infection and a CD4 count of 1184 cells/mm³ at diagnosis.
- Three years later, viral load increased from 3.5 to 5.7 $\log_{10}$ copies/ml. He was asymptomatic.
- Phylogenetic analysis suggested infection with a divergent virus strain, which did not show drug-resistance mutations.
- Over the subsequent year, the viral load returned to 3.5 $\log_{10}$ copies/ml, whereas the CD4 count remained $>1000$ cells/mm³ in the absence of antiretroviral therapy.
a) Maximum likelihood tree drawn in PhyML3.0 with *int* population sequence  
b) Maximum likelihood tree drawn in RAxML with *gag* and using 243 subtype B reference sequences from Los Alamos database.

Presented at the 8th European HIV Drug Resistance Workshop, March 17-19 2010, Sorrento, Italy
Other findings

- Initial screening suggested superinfection in 3 other patients, including 2 with possible evidence of recombination.
- Further analysis of pol, gag, and integrase excluded the events.
Conclusions

- 4% (2/47) HAART-naïve MSM showing a viral load increase of $\geq 0.5 \log_{10}$ cps/ml during routine clinical monitoring had evidence of superinfection.
- Viral load increases are common, but superinfection is not a common cause in MSM.
- The superinfecting strains can outgrow the previously established virus and become established as the dominant replicating species in peripheral blood.
- Superinfection can be associated with a recurrence of symptoms of primary infection or occur in the absence of recognised symptoms.
- HIV-1 infected patients who engage in high-risk sexual behaviour are at risk of superinfection both in the early and established phase of the disease.
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