Feasibility and clinical utility of HIV-1 genotype testing in the setting of low level viremia

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Background

Resistance testing in HIV-1 infected patients is recommended to guide the choice of antiretroviral therapy in clinical practice.

In this regard, genotypic testing is the most common method used for detecting drug-resistant strains of HIV. *(Vandamme AM, et al. AIDS Rev 2011; IAS-USA guidelines, July 2012; EACS Guidelines, October 2013; DHHS Guidelines, February 2013)*

This assay is usually not recommended in persons with a plasma **viral load <500-1,000 copies/mL**.
(DHHS Guidelines, February 2013; EACS Guidelines, October 2013)

Objective

We evaluated reliability and clinical usefulness of genotypic resistance testing (GRT), in patients failing combination antiretroviral therapy (cART) with viremia levels 50-1,000 copies/mL.

Materials and Methods

A retrospective analysis was performed on **13,684 HIV-1 clinical samples** that were genotyped over the **years 1999-2013** in two clinical centers in Rome (Italy), by means of **ViroSeq HIV-1 Genotyping System** (Abbott Molecular) or of a **Home–made system**.

Samples were **stratified in 6 groups according to different viremia levels** (**50-200**; **201-500**; **501-1,000**; **1,001-10,000**; **10,001-100,000**; **>100,000** copies/mL).

Genotyping success rate was determined in total and per each viremia group, independent of the genotyping platform upgrades (equipment, kits and reagents) that occurred during this period.

Phylogenetic analysis

In order to test genotyping **reliability** with viremia ≤500 copies/mL, a **phylogenetic analysis** was performed on **1,613 sequences** from **470 patients** with:

- **at least 1 sequence with contextual viremia ≤500 copies/mL**
- **at least 1 sequence with viremia >1,000 copies/mL**

Evaluation of resistance in patients failing therapy

The **relationship** between **different viremia levels** at the **time of the genotyping** and the **detection of major resistance mutations** (MRM, *IAS list 2013*) was evaluated in a subset of **3,985** samples successfully genotyped from **2,200** patients who had failed therapy (virological failure defined as viremia >50 copies/mL).

We evaluated:

- The **resistance to any drug-class** in the **overall samples analyzed**
- The **resistance to specific drug-class** among samples from:
  - patients who received **regimens** that **contained NRTIs**
  - patients who received **regimens** that **contained NNRTIs**
  - patients who received **regimens** that **contained PIs**

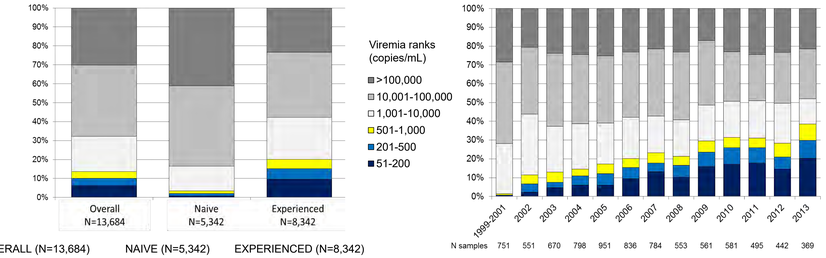
Potential differences in the prevalence of resistance among the **different viremia groups** were evaluated both by **Chi-Squared test for trend** (by comparing all the viremia groups) and **Chi-Squared test/Fisher's exact test** (by comparing two viremia-groups at a time).

Multivariable logistic regression analyses were used to evaluate i) **the impact of viremia levels at genotyping on the prevalence of drug-resistance**; ii) **the impact of pre-HAART viremia on the prevalence of drug-resistance in samples from patients at first line failure.**

Results

An increased number of GRTs have been requested over the years for patients failing with low viremia values.

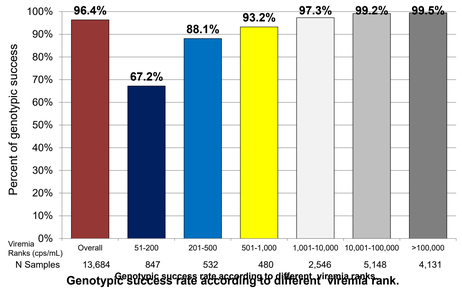
Among the overall genotypic requests for samples with viremia >50 copies/mL, **1,379/13,648 (10%)** were from **viremia levels ≤500 copies/mL.** In **experienced patients, 1,269 (15%) samples were with these viremia levels.**



Overview of 13,648 genotypic requests over the years from clinical samples with different viremia ranks starting from levels >50 copies/mL. Update to October 2013.

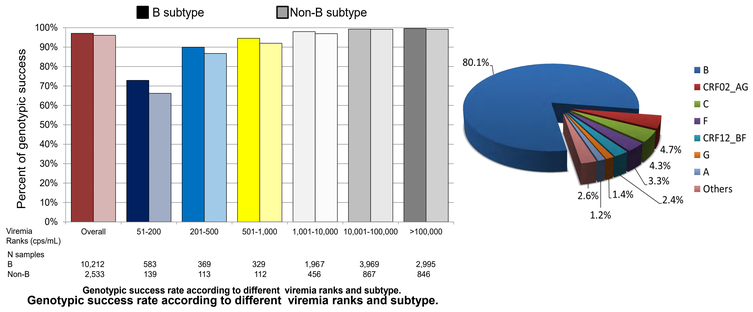
8,342 genotypic requests from plasma samples of treatment experienced patients stratified by viremia and years. Update to October 2013.

Overall, the genotyping success rate was 96.4%, and was independent from subtype in all viremia groups.



Genotypic success rate according to different viremia rank.

Genotyping success was ≥88.1% above 200 HIV-RNA copies/mL. A quite reasonable chance of success was obtained also between 50 and 200 copies/mL.



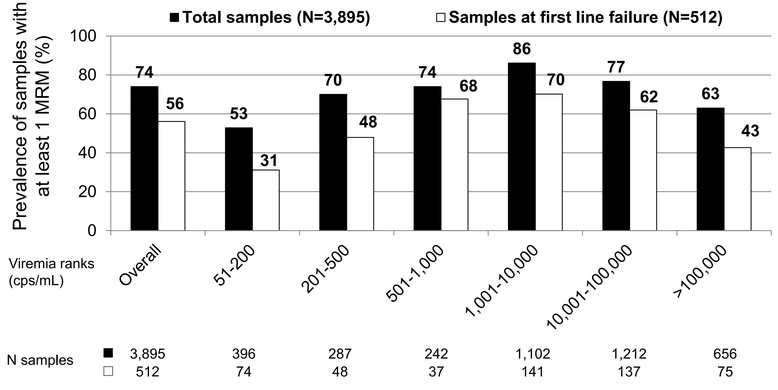
Genotypic success rate according to different viremia ranks and subtype.

▪**Samples** analyzed were **80.1% from** patients carrying a **subtype B virus**, and **19.9% from** patients carrying **subtype non-B viruses**. The following **non-B subtypes** were the **most prevalent**: **CRF02_AG** (**4.7%**), **C** (**4.3%**), **F** (**3.3%**).

Phylogenetic analysis (on 1,613 sequences) revealed a high homology within the sequences performed from the same subject for 453/470 (96.4%) patients analyzed, also for sequences with viremia levels <100 copies/mL.

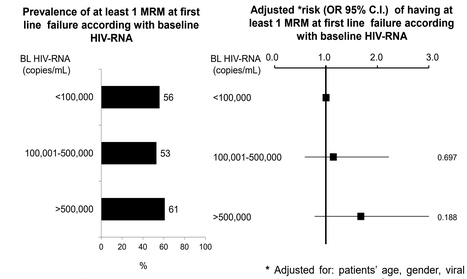
The overall prevalence of samples with at least one major resistance mutation (MRM) from treated-patients was 74% in total and 56% in samples from patients at first line failure, respectively. Of note, a considerable resistance was observed also at low levels of viremia.

The prevalence of resistance significantly varied according to the viremia levels (P<0.001). **The distribution of drug-resistance stratified for viral load was confirmed also considering samples only from patients failing their first-line regimen**, though with a lower prevalence.



8,342 genotypic requests from plasma samples of treatment experienced patients stratified by viremia and years. Update to October 2013.

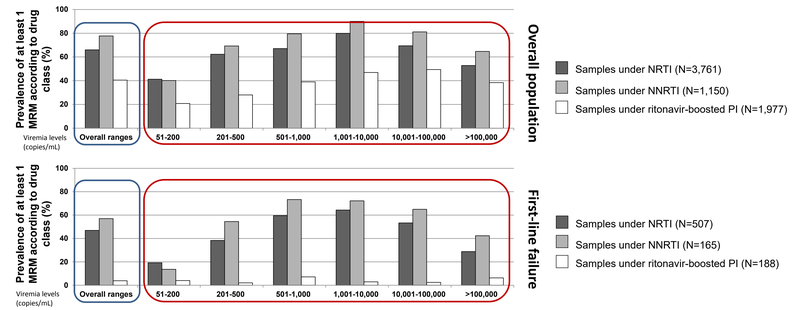
A trend toward significance in the relation between pre-HAART viremia and the prevalence of resistance at first line failure was found.



P=0.448 (Chi-squared test for trend)

* Adjusted for: patients' age, gender, viral subtype, calendar year of genotyping, and third drug (PI vs. NNRTI). BL: baseline

PI-resistance was significantly less frequent than NRTI/NNRTI-resistance in all viremia levels (P<0.001), especially at first-line failures (overall prevalence: 3.8%).



Proportion of resistance to NRTI, NNRTI, and PI classes in patients treated with NRTIs, NNRTIs and boosted PIs, respectively. MRM: major resistance mutation.

Multivariable logistic regression confirmed that the risk of having drug-resistance is associated with viremia levels at GRT (P<0.001).

Adjusted[a] Odd ratio (95% CI) to have at least 1 major resistance mutation (MRM) according to plasma viremia ranges at failure

Viremia ranges (copies/mL)	Any MRM[b]	NRTI MRM[c]	NNRTI MRM[d]	PI MRM[e]
51-200	<0.001	<0.001	<0.001	<0.001
201-500	<0.001	<0.001	<0.001	<0.001
501-1,000	<0.001	0.003	0.029	0.526
1,001-10,000[f]				
10,001-10,0000	<0.001	<0.001	<0.001	0.037
>100,000	<0.001	<0.001	<0.001	<0.001

a: Adjusted for: patients' age, gender, viral subtype, calendar year of genotyping, number of regimes experienced at the date of sampling, and boosted-PI administration (this last variable was specifically included in the model for the PI resistance). b: Overall population (N=3,895). c: Samples under NRTI pressure at the moment of genotyping (N=3,761). d: Samples under NNRTI pressure at the moment of genotyping (N=1,150). e: Samples under PI (boosted or unboosted) pressure at the moment of genotyping (N=2,609). f: Reference group. Abbreviations: CI, confidence interval; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; OR, odds ratio; P, P value; PI, protease inhibitor.

Conclusions

In patients failing cART with low viremia levels (50-1,000 copies/mL), HIV-1 genotyping provides reliable and reproducible results, that are informative about emerging drug-resistance. Our findings emphasize the importance of using the genotypic test at the first treatment failures also at low viremia, to guide the choice of an effective alternative regimen and decreasing the risk of resistance accumulation.

Acknowledgments

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