Feasibility and Reproducibility of HIV-1 Genotype Resistance Test in Very-Low-Level Viremia

Bianca Bruzzone, Antonio Di Biagio, Laura Sticchi, Renata Barresi, Francesco Saladini, Giancarlo Icardi, Maurizio Setti

Hygiene Unit, IRCCS AOU San Martino-IST, Genoa, Italy; Infectious Diseases Unit, IRCCS AOU San Martino-IST, Genoa, Italy; Department of Health Sciences, University of Genoa, Genoa, Italy; Department of Medical Biotechnologies, University of Siena, Siena, Italy; Internal Medicine and Clinical Immunology Unit, IRCCS AOU San Martino-IST, Genoa, Italy

In a recent review, Ryscavage et al. (1) underlined the need to increase availability of assays to genotype samples with HIV-1 RNA levels of <400 copies/ml. In addition, the studies by Santoro et al. and Gonzalez-Serna et al. (2, 3) provide data to support reliability and usefulness of HIV-1 genotype resistance testing (GRT) in HIV-1 patients failing combined antiretroviral treatment (cART) with low-level viremia (LLV). These results are considered predictive of future virologic outcomes (3). With the present letter, we would like to give our contribution, reporting on the results of sequencing samples not only with LLV but also with very low-level viremia (VLLV).

From January 2013 to date, 439 consecutive samples tested for both HIV-1 RNA quantification and GRT have been evaluated. Here we report on the results obtained with the 168 samples with HIV-1 RNA quantified below 1,000 copies/ml by the Versant HIV-1 RNA 1.0 assay (kPCR) (Siemens HealthCare Diagnostics). HIV-1 RNA values of <50 copies/ml were confirmed by a Nuclisens EasyQ HIV-1 2.0 assay (bioMérieux SA). Sequences were obtained using a Trugene HIV-1 genotyping kit (Siemens HealthCare Diagnostics) after automated extraction (NucliSens EasyMAG; bioMérieux SA) of 1 ml of plasma and concentration of the eluate in 25 μl. The only change made to the manufacturers’ instructions was the addition of 20 μl of extract instead of 17 μl in the first amplification.

Samples were stratified into five groups according to the obtained HIV-1 RNA values (<50, 50 to 100, 101 to 200, 201 to 500 and 501 to 1,000 copies/ml), regardless of subtyping, which was B in 88.7%, followed by F (3.0%), CRF01_AE (1.8%), and others. Resistance-associated mutations (RAMs) for nucleoside reverse transcriptase inhibitors (NRTI), nonnucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI), according to the 2013 IAS list (7), were evaluated in each group.

The numbers of analyzed sequences, success rates of genotyping, and rates of resistance for the presence of at least one mutation and for each class of drug were assessed according to the stratifications described above and are shown in Table 1.

Very much in accordance with the cited studies, the overall success rate of genotyping was 168/180 (93.3%). Remarkably, the successful genotyping rate was very high (82.1%) even in samples with VLLV (i.e., <50 copies/ml). It is noteworthy that the RAM rate was relevant in all stratifications (overall, 39.3%), including the VLLV group (30.4%). The overall rates of resistance to NRTI, NNRTI, and PI were 27.4%, 23.2%, and 11.3%, respectively, and rates of resistance to 2 and to 3 classes of drugs were 5.9% and 7.7%. A phylogenetic analysis of 17 of 23 matched sequences of the samples with VLLV, for which a previous sequence was available when viremia was >1,000 copies/ml (Fig. 1), allowed us to rule out cross-contamination. Moreover, every other month the G02 OptiQual HIV-1 genotype control (AcroMetrix Corporation) and the negative and positive control of the Trugene HIV-1 genotyping kit are routinely performed in the laboratory.

These findings suggest that, when a virologic failure is suspected for even minimal increases of HIV-1 RNA values, GRT can be performed with a high rate of success even in samples with VLLV, thus both enhancing the chance of sequencing the mutated virus and increasing the possibility of protecting against the occurrence of supplementary mutations.

The reasons why guidelines indicate HIV-1 RNA limits of >500 copies/ml for GRT lie in the alleged inconsistent feasibility of testing and reproducibility of results below that level (4, 5). Only Italian guidelines recommend it for patients failing a cART with HIV-1 RNA values from 50 to 200 copies/ml (6). Our findings have the limitations of having been obtained from a relatively small series and with a kit that has been announced to be discontinued in the near future. However, by extending the results of other authors, they suggest that in clinical practice, the current

TABLE 1 Number of analyzed sequences, success rate of genotyping, and rates of resistance stratified according to HIV-1 RNA level

<table>
<thead>
<tr>
<th>RNA level (copies/ml)</th>
<th>No. of sequences analyzed</th>
<th>Success rate (%)</th>
<th>At least 1 mutation</th>
<th>NRTI</th>
<th>NNRTI</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>23</td>
<td>82.1</td>
<td>30.4</td>
<td>21.7</td>
<td>8.7</td>
<td>4.3</td>
</tr>
<tr>
<td>50–100</td>
<td>29</td>
<td>85.3</td>
<td>44.8</td>
<td>27.6</td>
<td>31.0</td>
<td>6.9</td>
</tr>
<tr>
<td>101–200</td>
<td>34</td>
<td>94.4</td>
<td>41.2</td>
<td>23.5</td>
<td>29.4</td>
<td>14.7</td>
</tr>
<tr>
<td>201–500</td>
<td>56</td>
<td>100</td>
<td>41.0</td>
<td>30.4</td>
<td>25.0</td>
<td>12.5</td>
</tr>
<tr>
<td>501–1,000</td>
<td>26</td>
<td>100</td>
<td>34.6</td>
<td>30.8</td>
<td>15.4</td>
<td>15.4</td>
</tr>
</tbody>
</table>
methods for performing GRT are robust and efficient enough to deserve consideration even in the case of LLV and VLLV samples.

ACKNOWLEDGMENTS

We thank the HIV/HCV Liguria Study Group (A. Alessandrini, V. Bartolacci, S. Boni, G. Cassola, G. Cenderello, P. De Leo, C. Dentone, M. Guerra, G. Mazzarello, G. Penco, C. Viscoli) and Nigro Nicola for his technical support.

REFERENCES


