Prevalence of antiretroviral drug resistance among treatment-naïve and treated HIV-infected patients in Venezuela

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An in-house, low-cost method was developed to determine the genotypic resistance of immunodeficiency virus type 1 (HIV-1) isolates. All 179 Venezuelan isolates analysed belonged to subtype B. Primary drug resistance mutations were found in 11% of 63 treatment-naive patients. The prevalence of resistance in isolates from 116 HIV-positive patients under antiretroviral treatment was 47% to protease inhibitors, 65% to nucleoside inhibitors and 38% to non-nucleoside inhibitors, respectively. Around 50% of patients in the study harboured viruses with highly reduced susceptibility to the three classical types of drugs after only five years from their initial diagnoses.

Key words: HIV - genotypic resistance - diagnostic - Venezuela

Around 1.6 million persons are estimated to be infected with human immunodeficiency virus type 1 (HIV-1) in Latin America, including some 110,000 in Venezuela (UNAIDS/WHO 2007). The most frequently isolated viral type in the region is subtype B, although other subtypes, like subtype F and BF recombinant forms, are found in Brazil and the South Cone (Morgado et al. 2002). Highly active antiretroviral therapy (HAART) has been provided free of cost to a total of more than 21,000 patients in Venezuela as of the end of 2007. Genotypic testing by commercial methods adds an additional expense to the high cost of HIV treatment and is performed in Venezuela only for priority cases. Therefore, limited information related to genotypic resistance profiles is available locally. The availability of in-house, affordable genotypic testing offers the opportunity to detect drug resistance mutations even before the start of treatment. Such testing would also allow assessment of the prevalence of primary resistance mutations in the population. The aim of this study was to evaluate genotypic resistance among a representative group of HIV-1-infected Venezuelan patients as well as to assess drug resistance mutation frequencies in a cohort of treated and drug-naïve patients by means of an in-house RT-PCR method.

A total of 179 HIV-1-infected Venezuelan patients were studied, of which 63 patients were naïve and 116 were receiving HAART (most of these later developed virological failure), all predominantly from the national capital Caracas. Signed, informed written consent was obtained in each case. This study was approved by the Instituto Venezolano de Investigaciones Científicas Bioethical Committee. Blood samples were collected between 2004-2007. Information about the most probable mode of transmission and the time of diagnosis of infection was available from 95 and 96 patients, respectively. Adherence information was obtained by a questionnaire designed to determine the number of antiretroviral (ARV) doses missed in the past two weeks, classifying adherence as: 90-100%, 1-2 doses missed; 70-90%, 2-3 doses missed; 50-70%, 3-4 doses missed and < 50%, more than four doses missed.

Viral RNA was extracted from plasma with commercial kits (QIAamp® Viral RNA Kit, QIAGEN, Germany). Around 1,500 nucleotides of the POL gene were amplified by RT and nested PCR, using the following primers: Prot05 (Persaud et al. 2000) and Poll (5’-gttccagattttaatgcttga-3’) for the first round and Prot15 (Persaud et al. 2000) and Pol 2 (5’-aattgctttgataatgattag-3’) for the second round. The amplification conditions were: one round of 95°C for 2 min, 29 cycles of 90°C for 1 min, 55°C for 30 sec and 72°C for 2 min, with a final extension of 7 min at 72°C and an annealing temperature of 50°C for the second round. PCR-purified fragments were sent to Macrogen Sequencing Service (Macrogen, Korea) for sequencing using 5’ primers Prot15 and POL2 and 3’ extra primers Sp6 (5’-agatatcagtacaatgtgct-3’), A35 (Devereux et al. 2000) and 3Prot2 (Fleury et al. 2003). Sequences were submitted individually to a genotype sequence algorithm (Stanford University HIV Drug Resistance Database, http://hivdb.stanford.edu/index.html).

Overall, 28 HIV-1 plasma samples were tested for genotypic resistance by the in-house method and by a commercial test (Trugene HIV-1/Opengene, Bayer Health Care Laboratories). The sequences obtained by both methods were submitted to the CPR Stanford algo-
TABLE I
Prevalence of primary drug mutations in HIV-infected treatment naïve patients (n = 63)

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Patients harboring virus n (%)</th>
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<tbody>
<tr>
<td>None</td>
<td>39/63 (62)</td>
</tr>
<tr>
<td>Primary and secondary</td>
<td>24/63 (38)</td>
</tr>
<tr>
<td>Mutations conferring drug resistance</td>
<td>7/63 (11)</td>
</tr>
</tbody>
</table>

Primary mutations:
P1: M46I 1 (1.6)
NRTI: K70R 1 (1.6)
M184V 2 (3.2)*
T215S 1 (1.6)
K219E 1 (1.6)
NNRTI: K103N 1 (1.6)
G190A 1 (1.6)*

a: higher occurrence than shown in the HIV data base (CPR Stanford algorithm) for treatment-naïve patients (p < 0.005).
P1: protease inhibitor; NNRTI: non-nucleoside analog reverse transcriptase inhibitor; NRTI: nucleoside analog reverse transcriptase inhibitor.

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mutations vary according to region, with low prevalence in Rio de Janeiro (1.4%) (Varella et al. 2007) and higher prevalence in Sao Paulo (6.5%) (Gonzalez et al. 2007). The results of primary resistance testing in Venezuela warrant the cost-benefit analysis of a routine, local, primary genotypic resistance surveillance program.

Genotypic resistance was evaluated in a cohort of 116 HIV-positive patients on ARV treatment. The cohort consisted mostly of males (91%), with an average age of 39.7 years and a predominance of males who have sex with males (MSM; 51/89, 57%). In the 45 patients for whom adherence to HAART could be assessed reliably, adherence was as follows: more than 90% adherence in 22 patients (49%), 70-90% adherence in 13 (29%) and less than 70% in 10 (22%). These results suggest that around 50% of the patients exhibited lower than optimal adherence. The category of resistance found was as follows: 47% with resistance to PIs, 65% to nucleoside analog reverse transcriptase inhibitors (NRTIs) and 38% to nucleoside analog reverse transcriptase inhibitors non-nucleoside analog reverse transcriptase inhibitors (NNRTIs). Of note, 33% and 18% of them harboured mutations associated with resistance to both types of reverse transcriptase (RT) inhibitors or to protease and RT inhibitors, respectively. The prevalence of ARV resistance was compared to that reported in the Stanford database (“Mutation prevalence according to subtype and treatment”). A high prevalence of some mutations associated with resistance to NNRTIs, particularly K103N (27%), was found in this cohort of patients, as compared to the prevalence for this particular mutation reported in the HIV database (18%). On the other hand, the prevalence of resistance to NRTIs and PIs was generally similar to that reported in the HIV database (Table II). For most of the drugs used by the patients in this study, a significant positive correlation was found between the use of a specific ARV drug and the detection of resistance to that drug. Mutations conferring resistance to a particular drug were more frequently found among patients with a history of receiving that drug. Drug resistance mutations detected were as follows: 42/68 3TC-treated patients vs. 17/49 not treated with 3TC (p = 0.001), 43/63 AZT-treated patients vs. 16/47 not treated (p < 0.001), 12/18 RTV-treated patients vs. 12/37 not treated (p = 0.035), 24/38 EFV-treated patients vs. 12/37 not treated (p < 0.001) and 26/33 ABC-treated patients vs. 28/84 not treated (p < 0.001).

The prevalence of mutations conferring resistance to ARV drugs found in treated patients was similar to those described recently in Chile for PIs and NRTIs (46% and 61%, respectively) and similar to the prevalence reported in Rio de Janeiro, Brazil (70% to NRTIs, 55% to NNRTIs and 45% to PIs) (Bongertz et al. 2007, Rios et al. 2007). This prevalence was also higher than that reported for Venezuela earlier this decade by Delgado et al. (2001). However, resistance to NNRTIs was significantly higher in Chile (84%) (Rios et al. 2007). The same prevalence of resistance to PIs and NRTIs has been reported in a large study from the USA (41% and 71%, respectively), whereas the prevalence of resistance to NNRTIs was significantly lower (25%) (Richman et
A relatively high prevalence (p < 0.05) (Table I) of two PI resistance mutations at codons 47 and 54, which confer decreased susceptibility to several PI drugs, has also been observed previously (Johnson et al. 2006). While the overall prevalence of mutations conferring resistance to NNRTIs found in the current study was not particularly elevated, the observed rate of the NNRTI resistance K103N mutation was rather high (27%). This result is similar to a recent report from Brazil (Couto-Fernandez et al. 2005). Moreover, several studies report an increased prevalence of this mutation over time (De Mendoza et al. 2007), which might be related to an increase in viral fitness (Capetti et al. 2005). Alternatively, this mutation might be maintained simply because it has no negative effect on viral fitness.

The number of patients harbouring viruses with susceptibility to only one class of drugs (PI inhibitors, NRTIs and NNRTIs) or to no type of drug was analysed according to the time of diagnosis of infection, for the patients for whom this information was available. In our study, most of the patients requesting genotypic resistance analysis had been diagnosed between five and nine years ago. Moreover, about 50% of 67 patients already exhibited highly resistant viruses (susceptible to only one class of drugs or to none of these drugs), after as little as five years from the initial diagnosis. Several factors might account for this observation: a relatively late detection of HIV infection, inappropriate use of HAART drugs, lack of availability of important ARV drugs in Venezuela or, finally, poor adherence to treatment. Indeed, a recent study showed that in Venezuela, 40% of patients receive a diagnosis of HIV-1 infection at a late disease stage (Bonjour et al. 2008). In addition, it must be noted that our cohort study was comprised mainly of patients who were already at a stage of virological failure. Nevertheless, these results emphasise the importance of the rational use of ARV drugs and of adequate counselling for HIV-infected patients in order to maintain adherence to HAART.

**REFERENCES**


### TABLE II

Prevalence of primary drug mutations in HIV-infected treated patients (n = 116)

<table>
<thead>
<tr>
<th>Mutation associated to resistance to PI: 55/116 (47%)</th>
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<tr>
<td>D30</td>
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<td>N(7)</td>
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<table>
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<tr>
<th>Mutation associated to resistance to NRTI: 75/116 (65%)</th>
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<td>N(28)</td>
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<table>
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<tr>
<th>Mutation associated to resistance to NNRTI: 44/116 (38%)</th>
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<tr>
<td>A98</td>
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</table>

The letters in each column correspond to the amino acid found in this position. Numbers under parenthesis correspond to the percent prevalence found in the patients studied. Bold and italic letters indicate higher and lower, respectively, occurrence than the one shown in the HIV data base for treated patients (p < 0.005). PI: protease inhibitor; NNRTI: non-nucleoside analog reverse transcriptase inhibitor; NRTI: nucleoside analog reverse transcriptase inhibitor.


