

Transmission of Drug Resistant Viruses in Recent HIV Seroconverters in Spain

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Background

Multiple studies of surveillance of drug resistance in recent HIV-1 seroconverters have been conducted in the USA and Europe over the last years. The overall prevalence of primary drug resistance is around 10-15%, with some differences among regions and time periods.

In Spain, studies conducted over the last decade demonstrated a steady decline in the rate of genotypic resistance among drug-naïve individuals with chronic HIV-1 infection until year 2000, which has remained stable since then.

Studies in recently HIV-1 infected persons are of interest considering the implications of primary drug resistance for designing first-line therapies. Moreover, it may provide a unique opportunity to monitor the spread of new HIV-1 variants within a region and to track the source of new infections.

Objective

To analyze the prevalence of drug resistance mutations, non-B subtypes and transmission events during episodes of acute infection in a relatively large population of HIV-1 seroconverters identified in Spain during the last eight years.

Patients

The proportion of genotypic drug resistance was examined in consecutive newly HIV-1 infected individuals seen since January 1997 to December 2004 in 11 different hospitals distributed across Spain.

The eligibility criteria for a subject to be enrolled in the study were an HIV-1 laboratory evidence of acute primary HIV-1 infection (detectable plasma HIV-RNA together with negative or indeterminate HIV antibody tests) or seropositivity for HIV-1 infection (reactive ELISA and Western blot), being negative in a previous test performed within the previous 12 months.

Methods

Genetic sequence analyses of both HIV reverse transcriptase (RT) and protease genes was carried out in all plasma specimens using an automatic sequencer (ABI Prism 3100, Celega Diagnostics, Madrid, Spain). For the purpose of this study, only major or primary drug resistance mutations listed in the latest guidelines from the International AIDS Society-USA Panel were recorded.

To determine the HIV-1 subtype as well as to explore possible transmission events during episodes of acute infection, 207 sequences from HIV population were compared with reference strains as well as among themselves.

28 sequences were aligned with HIV-1 group M reference sequences using the CLUSTAL W method (Bioinformatics Resource Project, National Center for Biotechnology Information, Bethesda, MD). Phylogenetic analyses were performed using the PHYLIP software package (version 3.52, J. Felsenstein, University of Washington, Seattle, WA).

Evolutionary distances were estimated with Dnadist (linear two-parameter method) and phylogenetic relationships were determined by Neighbor-joining method. Branch reproducibility of trees was evaluated using 1000 replicates and Consense.

To confirm transmission events during episodes of acute infection, the vif region from the *env* gene was additionally sequenced. As recommended, all samples were run separately and a new specimen from each patient was used to exclude cross-contamination.

Results I

A total of 166 recent HIV-1 seroconverters were identified. The main characteristics of the study population are recorded in Table 1. Overall, 55.7% were men, and 70% were infected through homosexual contacts. The mean estimated time of HIV-1 infection was 3 months.

Main plasma viraemia and CD4 counts at the time of the analysis were 4.6 HIV-RNA log copies/ml and 570 cells/mm³, ranged only

Table 1: Main characteristics of the study population.

No.	166
Sex	
Men (%)	63.2
Mean age (years)	31
Risk group (%)	
Homosexual men	70
Heterosexual	19.5
Intravenous drug users	10
Blood transfusion	0.5
Median time from infection (months)	3 (1-11)
Median CD4 count (cells/mm ³)	570 (403-734)
Median viral load (log ₁₀ HIV-RNA copies/ml)	4.63 (4.14-5.1)

Results II

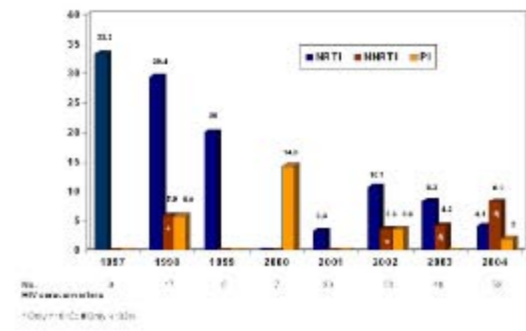
The overall rate of drug-resistant viruses in this population was 12.1%. By drug families it was 9.8%, 4.1% and 2.1% for nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI), respectively (Table 2).

By calendar year 33.3% (1997), 25.4% (1999), 29% (2001), 14.3% (2001), 3.7% (2002), 10.4% (2003) and 8.2% (2004) (Figure 1).

Table 2: Prevalence of HIV drug resistance mutations in HIV recent seroconverters from 1997 to 2004.

Year	N	NRTI (%)	NNRTI (%)	PI (%)
1997	33	33.3	0	0
1999	31	25.8	0	0
2001	34	29	0	0
2002	3	0	0	0
2003	21	14.3	0	0
2004	21	8.2	0	0
Total	166	12.1	0	0

Figure 1: Yearly proportion of viruses with resistance mutations to the different antiretroviral drug classes during the study period.



Results III

A linear by linear association showed a significant reduction in the overall rate of drug resistance among recent HIV-1 seroconverters ($p=0.010$). This was mainly driven by a significant decline in NRTI resistance mutations over time ($p=0.001$).

By contrast, a significant increase in the rate of NNRTI resistance mutations was seen in recent years, mainly driven by K103N. Vif in this mutation was absent before year 2002, it risen to 4.2% in 2003 and to 2.2% in 2004 ($p=0.02$).

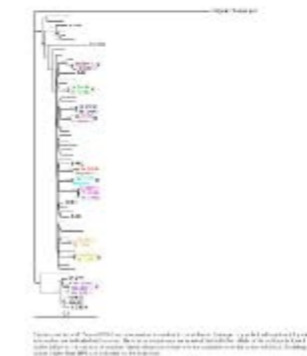
Drug resistance to PI remained relatively stable during the whole study period, with identification of mutations at positions 41, 63 and 90 as the main protease resistance transmitted.

A total of 15 individuals (7.6%) were infected with HIV-1 non-B subtypes, distributed as follows: 7 CRF14_BG, 2 CRF_2_C, 2 CRF_2_C and 2 CRF14_AG. Interestingly, the recognition of all these non-B variants was restricted to the last 3 years. Moreover, a significant association between infection with non-B subtypes and acquisition of HIV-1 through intravenous drug use (IDU) (33.3%, $p=0.001$) and heterosexual contact (11.4%, $p=0.02$) was observed with respect to seroconversion relationships (1.6%).

CRF14_BG viruses were identified in two IDUs and one individual infected by heterosexual contacts. Among the latter, one was a Spanish man who admitted multiple partnerships, including IDUs, and another was a Portuguese woman with a female sexual partner from Viana do Castelo known to be HIV-1 seropositive.

Overall, subtype B represented 83% of viruses involved in these clusters, 72% of which occurred among homosexual men. Interestingly, one cluster occurring among other risk groups involved three IDUs, all of whom acquired CRF14_BG during an interval of 4 weeks.

Figure 2: Phylogenetic tree based on *pol* sequences belonging to individuals with recent HIV-1 infection and possible transmission contacts.



Conclusion

The overall rate of drug resistance among recent HIV-1 seroconverters in Spain is currently around 10%. Three aspects merit particular attention:

- First, resistance to non-nucleoside reverse transcriptase inhibitors is increasingly being transmitted with respect to resistance to other antiretroviral drug classes.
- Second, transmission of non-B subtypes is rising, and the new CRF14_BG strain is spreading among newly infected drug users and their heterosexual partners.
- Third, there is evidence of transmission during episodes of acute infection, suggesting that prevention strategies should be reinforced in risk populations.