CONCISE COMMUNICATION

Performance of drug resistance assays in testing HIV-1 non-B subtypes

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Antiretroviral susceptibility analyses were performed in plasma samples collected from 32 HIV-1 non-B-infected individuals, most of whom had received antiretroviral drugs. Reverse transcriptase (RT) and protease gene sequences were obtained, and 15 anti-HIV drugs were tested in a recombinant virus phenotypic assay. Phenotypic results were obtained in 25 (78.1%) samples, while genotypic data were recorded in 19 (59.4%). In seven samples (21.9%), neither genotypic nor phenotypic results were obtained. Ten of 13 samples with plasma HIV RNA below 2000 copies/mL did not yield genotypic results. Resistance assays work accurately when testing HIV-1 non-B subtypes. However, as for subtype B variants, a low viral load is the most important factor limiting the application of these tests.

Keywords Subtypes, HIV-1, drug resistance, genotype, phenotype, viral load

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INTRODUCTION

Drug resistance testing has become an important tool in the management of HIV-infected individuals, mainly in those undergoing antiretroviral therapy [1–3]. Several assays have been developed for recognizing drug-resistant genotypes in clinical samples [4–6]. Likewise, different phenotypic tests are now available [7,8]. Genotypic assays are the most widely used, since they provide results more rapidly and are cheaper than phenotypic tests. However, the efficiency of these assays seems to be compromised when testing specimens with low viral load, a circumstance which limits their application in early virologic failures under antiretroviral therapy [3,9]. A second limitation of these assays—so far unexplored—could be deduced from the genetic heterogeneity in the HIV genome, particularly if genomic changes exist at sites involved in primer binding. The prevalence of HIV-1 non-B variants seems to be increasing in North America [10,11] and Europe [11–13]; therefore, the performance of drug resistance assays in the testing of non-B variants needs to be assessed before they enter the market.

Automatic population-based sequencing is currently the most widely used approach for drug resistance genotyping. Most current phenotypic tests are based on recombinant virus assay technology [14–16]. Some of these tests are now commercially available, and widely implemented in clinical sites. In this study, we have analyzed the performance of representative genotypic and phenotypic technologies in the examination of resistance to both reverse transcriptase (RT) and protease inhibitors in clinical samples from subjects carrying HIV-1 non-B subtypes.

PATIENTS AND METHODS

Plasma samples from 32 HIV-1 non-B subtype-infected individuals were examined for the presence of drug resistance using commercial genotypic and phenotypic assays. Infected subjects were foreigners living in Spain, mostly coming from African countries (28/32; 87.5%), undergoing regular follow-up in an HIV/AIDS clinic in Madrid. One-third of them were receiving antiretroviral therapy at the time blood was drawn. The genetic characterization of HIV-1 subtypes infecting those individuals has been reported previously [12,17,18]. Although most individuals carried subtype G viruses ($n = 12$), subtypes A, D, F and H were also represented (one each). Fourteen

subjects carried recombinant forms (mostly A/G), and two had non-B variants with no clear affiliation. However, the genetic distances of these non-B variants with respect to subtype B reference isolates were high enough to confirm them as subtype non-B.

Plasma viral load was measured using the second-generation bDNA assay (Quantiplex v2.0, Bayer, Barcelona, Spain), which has a detection limit of 500 HIV RNA copies/mL.

Plasma HIV RNA extraction, cDNA synthesis, PCR amplification, purification for genetic (VircoGEN) and phenotypic (Antivirogram) assays were all performed as previously described [16]. Briefly, the VircoGE assay is a genotypic resistance assay which examines sequences from the *gag* region, the complete protease-coding region, and the first 400 amino acids of the RT gene. A 'virtual' phenotype can be generated from the VircoGE assay by use of a software program based on algorithms developed using the unique correlation database available at VIRCO, which links paired phenotypic and genotypic data in more than 3000 clinical plasma samples [19]. The Antivirogram uses a rapid new-generation recombinant virus assay [16]. It gives real as opposed to inferred *in vitro* phenotypic results.

RESULTS

Two thirds of the samples ($n = 21$) had plasma viral load values above 2000 HIV RNA copies/mL. The genotypic assay (VircoGEN) yielded results in 19 (59.4%) of the tested specimens, while phenotypic data were recorded in 25 (78.1%). The Antivirogram provided phenotypic information in all samples yielding genotypic results (Table 1). In seven samples (21.9%), neither genotypic nor phenotypic information could be obtained. Plasma viral load was below 2000 HIV RNA copies/mL (range: 50–1575) in all of them.

Table 2 summarizes the results obtained by the genotypic and phenotypic assays with respect to the different HIV-1 subtypes examined. No specific subtypes were missed more frequently than others, although this aspect could not be addressed appropriately, since most specimens belonged to subtype G and/or were recombinant forms, the remaining subtypes being not well represented. Eighteen (85.7%) of the 19 samples which could be genotyped and phenotyped had plasma viral load values above 2000 RNA copies/mL. The single sample yielding positive results by these assays, but having a viral load below 2000 HIV RNA copies/mL, was from a subtype G-infected African individual. Plasma viremia in this patient was below the detection limit using the bDNA, as well as other quantitative methods, such as NASBA and Amplicor.

The comparison between virtual (inferred from viral genotype) and real phenotypes was quite concordant. In 10 of 19 individuals for whom genotypic information was obtained, a wild-type phenotype was noted and no mutations were found in the RT and protease genes. In contrast, in five subjects, the presence of genotypic changes was associated with the presence of phenotypic resistance, either real or virtual. In one of them, virtual resistance to indinavir/ritonavir was not confirmed with the recombinant virus assay,

Table 1 Results obtained in drug resistance assays according to plasma viral load values

Viral load (HIV RNA copies/mL)	Genotype (VircoGEN		Phenotype (Antivirogram)	
	Yes ($n = 19$)	No ($n = 13$)	Yes ($n = 25$)	No ($n = 7$)
>2000 ($n = 21$)	18	3	21	0
<2000 ($n = 11$)	1	10	4	7

Table 2 Performance of genotypic and phenotypic drug resistance assays in respect to different HIV-1 subtypes

Genotype (Vircoge)	Phenotype (Antivirogram)	Samples		Subtypes
		No.	%	
Yes	Yes	19	59.4	8 G, 8 RF, 1 D, 1 F, 1 NA
No	Yes	6	18.7	2 G, 3 RF, 1 H
No	No	7	21.9	2 G, 3 RF, 1 A, 1 NA

NA, not assigned to a subtype; RF, recombinant forms.

which indicated an intermediate level of resistance to non-nucleoside analogs. In four samples, an apparent discordance between the virtual and real phenotype emerged, three of them being considered as resistant by the real phenotype but sensitive by the virtual interpretation. In another, virtual resistance to AZT (inferred from sequence data) was not confirmed using the recombinant virus assay.

DISCUSSION

The increasing global spread of HIV-1 distinct subtypes highlights the need to determine genotypic drug resistance in subtypes other than subtype B [10,11]. Assays determining the drug susceptibility of HIV-1 isolates should ideally be rapid, reproducible, and applicable to all HIV-1 variants, including all HIV-1 subtypes. However, most of the current resistance tests have been designed on the basis of HIV-1 subtype B strains, and little is known of their performance in testing non-B clades. HIV-1 genotyping methods are faster and less complex than phenotyping, but their efficiency could be limited when testing viruses with high genetic diversity. First, this could be due to misrecognition of primer binding sites, yielding negative results for amplification, as has been noticed for viral load quantification tests [20]. Second, limitations might arise from the presence of complex genomes with uncertain interpretations, such as drug resistance mutant combinations, mixed viral populations, or accessory nucleotide changes not associated with drug resistance. The real phenotype, obtained after confronting the tested virus with different drug concentrations, should be of great value in these circumstances.

Overall, in our study, HIV-1 non-B subtypes could be genotyped in 59.4% of specimens, although phenotyping was provided in up to 78.1% of specimens. A low viral load was the main factor associated with a lack of results, either genotypic or phenotypic. No specific subtypes were missed more frequently than others, although this aspect could not be addressed appropriately in our study, since many specimens belonged to subtype G and the rest were not well represented.

In one individual, genotypic and phenotypic results could be obtained despite the viral load being below the limit of detection in three different assays. In this sample, the virtual and real phenotypes showed resistance to nevirapine (8.9-fold), and the CD4 count was declining under a triple combination which included nevirapine. Therefore, viral replication most likely occurred in this subject, and misrecognition of plasma viremia occurred with the current viral load assays, as has been previously noticed when testing some non-B subtypes [20]. Apparently, the primers used in the VIRCO drug resistance tests did not target the viral genome sufficiently well in this sample. Conversely, three specimens could not be genotyped by the VircoGEN assay, despite their harboring more than 2000 HIV-1 RNA copies/mL according to the bDNA quantification assay.

The accuracy and reproducibility of the Antivirogram and VircoGEN assays were considered acceptable in a previous report [19]. However, in our study, the virtual and real phenotypes were not always concordant. The presence of minor resistant populations may be missed more often in the virtual phenotype and could explain the discordance. Moreover, new mutational patterns could explain the recognition of unexpected resistance phenotypes.

In conclusion, the performance of the VIRCO genotypic and phenotypic assays seems to be acceptable for testing samples from subjects infected with HIV-1 non-B subtypes. A low viral load appears to be the most important factor limiting the performance of these tests, for testing either HIV-1 B or non-B clades.

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