Clinical Utility of Genotypic Resistance Tests for HIV-1-Infected Patients with Low-Level Virological Failure


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Received 30 June 2010/Accepted 6 July 2010

The usefulness of genotypic resistance tests (GRT) among HIV-1 patients with low-level virological failure (LLVF) was evaluated. Up to 78% of samples with <1,000 copies/ml were sequenced successfully. For samples with 50 to 200 copies/ml, the success rate was as high as 69%. LLVF should not deter clinicians from requesting GRT.

The goal of highly active antiretroviral therapy (HAART) in HIV-1-infected patients is the suppression of plasma viral load to <50 copies/ml (1–3). The emergence of drug resistance is one of the main challenges. Conventionally, viral loads of >1,000 copies/ml were considered to be necessary for the success of sequencing-based genotypic resistance tests (GRT) (7). However, as HIV-1 is known to develop resistance even at low-virological failure (LLVF) (4, 5, 8), early identification of resistance is paramount. We assessed the clinical utility of performing GRT among patients on HAART with viral loads of <1,000 copies/ml.

All GRT requests to the virology laboratory at Guy’s and St. Thomas’ Hospital in South London, United Kingdom, for treatment failure in adult HIV-1-infected patients between January 2005 and June 2007 were analyzed. Treatment failure was defined as a rebound of viral load of >50 copies/ml on two separate occasions (virological rebound) or failure to achieve <50 copies/ml within 6 months of initiation or switching of HAART (failure to suppress). Repeat samples taken within 6 months of the first sample were excluded from the analysis. Patients with more than one episode of treatment failure occurring at least 6 months apart were analyzed as different episodes.

Two GRT, the Trugene HIV-1 genotyping assay (Trugene; Bayer HealthCare, Tarrytown, NY) and the Virco Type HIV-1 assay (Virco; VIRCO BVBA, Mechelen, Belgium), were used during the study period. Trugene was performed on-site once every 2 weeks, and Virco was performed at another laboratory. The choice of test was dependent on which of the two assays would provide the fastest turnaround time for results.

The Trugene assay was performed according to the manufacturer’s instructions as previously described (6). One milliliter of plasma was used for samples with a viral load of <1,000 copies/ml, and 140 μl was used with viral loads of >1,000 copies/ml. The target sequence included the majority of the protease (PR) (codons 4 to 99) and reverse transcriptase (RT) (codons 38 to 247) genes. The Virco assay is a service provided by the Virco laboratory (Mechelen, Belgium), and 1 ml of plasma was submitted for each request. The complete 99 codons of the PR gene and the 400 codons of the RT gene of HIV-1 were determined by standard DNA sequencing. Raw sequence data were submitted online to the University of Stanford’s HIV Drug Resistance Database (HIVdb Program; http://hivdb.stanford.edu) for analysis of resistance-associated mutations (RAM). Patients were stratified as high-level virological failure (HLVF) or LLVF, as defined by viral loads of ≥1,000 copies/ml or <1,000 copies/ml, respectively, at the time of GRT. The successes of the GRT, the resistance profiles, and the virological outcomes 6 months after GRT were compared.

One hundred eighty-eight treatment failure episodes from 158 patients were investigated. The HLVF group (n = 110) had more failure to suppress than the LLVF group, whereas the LLVF group (n = 78) had more virological rebound (Table 1). Of the LLVF samples, 22 were tested by Virco and 56 by Trugene; of the HLVF samples, 64 were tested by Virco and 46 by Trugene.

In total, 6 HLVF and 17 LLVF failed GRT (P = 0.0008). Virco had 10 failures (7 LLVF and 3 HLVF), whereas Trugene had 13 (10 LLVF and 3 HLVF). The median viral loads of the failed LLVF samples were 191 and 199 copies/ml for Virco (range, 54 to 670 copies/ml) and Trugene (range, 94 to 484 copies/ml), respectively. There was no significant difference in the success rates between Virco and Trugene at either HLVF or LLVF. However, there was a statistically significant difference in the trends for successful sequencing, with a progressive decrease in successful sequencing for samples with viral loads of >1,000, 201 to 1,000, and 50 to 200 copies/ml from 95% to 87% to 69%, respectively (P = 0.0005). Despite this difference, 78% (61/78) of LLVF samples were sequenced successfully. There was no significant difference in the frequencies of detection of RAM between the LLVF and HLVF samples. There was also no significant difference when the samples were analyzed according to the number of drug classes with resistance (Table 1).

Six months after the GRT, 7 patients (1 LLVF, 6 HLVF)
were lost to follow-up. Excluding these, 41 of 60 LLVF (68%) and 42 of 98 HLVF (43%) patients achieved a viral load of <50 copies/ml (P = 0.0019). Considering those lost to follow-up as having viral loads of >50 copies/ml in an intention-to-treat analysis, 41 of 61 (67%) LLVF patients and 42 of 104 (40%) HLVF patients achieved viral loads of <50 copies/ml within 6 months after GRT (P = 0.0009).

It is well recognized that viral load is a significant factor in determining the success of sequencing-based GRT (4, 6, 8), and results from this study support this. Samples with viral loads of <200 copies/ml were significantly less likely to have a successful GRT. Nevertheless, a large proportion (69%) of these samples were successfully sequenced. The two sequencing methods used in this study seem to be equally effective amplifying LLVF samples as they are amplifying HLVF samples.

In this study, patients with LLVF were more likely to have viral loads of <50 copies/ml than those with HLVF 6 months after the GRT. This could be due to more effective interventions taken at an earlier stage of treatment failure. Other factors, such as better adherence to treatment in the LLVF group, may also be important.

The limitations of this study were its retrospective nature and the fact that the assignment of sequencing method was not prospectively randomized. However, as the decision on the choice of the assay was based on the timing of the batches being run and was not related to the viral load and patient characteristics, there should not be significant bias. Also, no significant difference was found between the two methods, suggesting that in the modern era of good standardized nucleic acid extraction and sequencing techniques, routine sequencing of samples with low-level viral loads is highly feasible and testing should not be restricted to samples with viral loads of >1,000 copies/ml. However, at low plasma HIV-1 RNA levels, it is possible that sequences were generated from one or very few HIV-1 RNA molecules and may not be fully representative of the in vivo population. Caution should therefore be exercised when interpreting sequence data from LLVF samples.

We recommend the routine use of GRT for HIV-1-infected patients even with LLVF. This allows earlier recognition of resistance to antiretroviral agents, informs earlier therapy change, and is likely to prevent accumulation of mutations and preserve future treatment options.

REFERENCES