Assays for monitoring HIV therapy in low-middle income countries

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Abstract

The purpose of this thesis was to develop and/or evaluate assays for HIV viral load (VL) monitoring and HIV drug resistance testing of potential importance for research studies and clinical care in low- and middle income countries (LMICs). This was achieved using reverse transcriptase based (RT) assays and blood samples from Vietnamese and Swedish HIV-1 infected patients.

In Paper I, HIV-1 obtained from 63 treatment-naïve Vietnamese patients was analysed by population sequencing and phylogenetic analysis with regard to transmitted drug resistance (TDR), subtype and the time of the most recent common ancestor (tMRCA). All strains belonged to HIV-1 CRF01_AE and TDR was found in 6.3% of the patients, including Y181C, L74I, V75M and L210W mutations. tMRCA was found to be 1989.8 for a larger clade and 1997.5 for a smaller clade. Sequences from intravenous drug addicts were intermingled with sequences from sexually infected patients, indicating frequent exchange of virus between the transmission risk groups. Our data suggests that TDR and the transmission patterns between risk groups rate should be monitored regularly and prospectively.

In Paper II, we evaluated the feasibility, sensitivity and specificity of an RT-based assay for quantification of HIV. A high correlation ($r^2 = 0.97$), agreement ($\log$ difference = 0.34; 95% CI -0.35;1.03), sensitivity (98%) and specificity (100%) were found between the RT-based assay and the Roche Cobas TaqMan. Its feasibility was further confirmed in a clinical trial including 605 Vietnamese HIV-1 infected patients. Our results show that the RT-based assay is an attractive low-cost alternative for monitoring of efficacy of antiretroviral therapy programs in resource-limited settings.

In Paper III, a simple phenotypic RT-based assay was developed for the detection of drug resistance to the 2nd generation NNRTI etravirine (ETR) and cross-resistance patterns to the 1st generation NNRTIs. For all recombinant HIV-1 RTs, ETR displayed expected IC$_{50}$ values equivalent to previous reports. The test could detect ETR resistance in plasma samples (n=28) obtained from treatment-naïve and experienced Swedish HIV-1 infected patients associated with Y181C and L100I substitutions as well as discriminate between the impact of K103N on the IC$_{50}$ value of nevirapine but the lack of impact on the IC$_{50}$ value of ETR. In Paper IV, a further comparison was performed between our phenotypic ETR resistance assay and the genotype obtained by direct sequencing and ultra-deep pyrosequencing (UDPS) in 20 Swedish patients with past or ongoing failure on the 1st generation NNRTIs. Most of the strains from the patients had various degrees of decreased phenotypic ETR susceptibility despite absence of ETR resistance associated mutations (RAMs) according to direct sequencing. Additional resistance mutations corresponding to <20% of the viral populations were found by UDPS in 9 analysed patients. In four of these, the mutations are likely to have contributed to phenotypic resistance. The patient treatment histories and the UDPS data supported that our phenotypic assay may be more sensitive than direct sequencing in identifying minor quasispecies with resistance mutations. The degree and pattern of an increased assay sensitivity as well as the clinical relevance remains to be determined.

In Summary, since the evaluated RT-based assays are simple to perform, use basic laboratory equipment, and does not require complex interpretations, they could be a low cost alternative for both studying VL and drug resistance to 1st and 2nd generation NNRTIs in LMICs.