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Short Communication

Efficacy of genotypic drug resistance testing in patients with low-level plasma HIV-1 viremia

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ABSTRACT

Background: Drug resistance is common at low HIV viral loads and often requires modification of antiretroviral treatment, however commercial genotyping platforms are optimized for viremia >2000 copies/ml. Our aim was to analyze the results of drug resistance genotyping in samples with viral load <2000 copies/ml.

Findings: Resistance testing was performed using combined commercial (Viroseq 2.8) and in-house bulk sequencing methodology in 18 treatment naive and 45 antiretroviral treated patients (in total 71 samples were analyzed). Drug resistance was evaluated based on the International AIDS Society-USA 2013 update. Sequencing success rate and drug resistance patterns were evaluated.

Overall sequencing success rate was 87.4%. In viremia range of 50–1000 copies/ml 86.6% samples were successfully sequenced with 75.0% for levels of 50–200 copies/ml, 90.5% for 201–500 copies/ml, 91.7% for 501–1000 copies/ml and 88.46% for 1001–2000 copies/ml. Drug resistance mutations were found in 43.5% sequences, the overall resistance prevalence for NRTI was 30.65%, for NNRI: 19.35% and 11.29% for PI. In treatment naive patients 31.25% sequences with drug resistance mutations were found, among antiretroviral treatment failing patients in 52.17% samples. Across viremia ranges resistance was observed in 55.6% sequences for 50–200 copies/ml, 36.84% for 201–500 copies/ml and 36.36% for 501–1000 copies/ml and 47.83% for 1001–2000 copies/ml.

Conclusions: Success rate for genotyping at low plasma viremia levels is high and allows to guide treatment optimization. As drug resistance is commonly found at low viral loads, genotyping should be attempted in every virologically failing patient regardless the plasma viremia.

1. Introduction

With virological failure defined as viremia >50 copies/ml across most of the treatment guidelines therapy optimization in patients with plasma viral load <1000 copies/ml (low-level viremia, LLV) currently requires resistance testing [1]. It was shown that drug resistance mutations are commonly found at low viral loads in treatment experienced patients. Additionally, in the clinical practice modification of the antiretroviral treatment based on the genotyping result has been proven beneficial [2]. Among patients who persistently fail antiretroviral treatment with viral load <1000 copies/ml the resistance is not only frequent but also diverse – not limited to the single drug class [3]. Significant proportion of individuals with low-level viremia on combined antiretroviral therapy (cART) progress to virologic failure [4] and emergence of new drug resistance mutations was observed across all drug classes in the number of reports and trials [5–7]. As a result accumulation of mutations may limit therapeutic options, even if immunologic reconstitution is observed [8]. Controversies related to the significance of the LLV are related to the fact that most of the circulating virus is probably a result of replication in the long-lived cells and majority of patients virologically re-suppress without treatment change. Re-suppression was observed in 76% of patients with LLV episodes in a study by Li et al., while in ACTG A5142 and A5095 trials it was observed in 65% of patients with accumulated drug resistance and 74% in cases without evidence of new resistance [6,7,9]. Resupression is most likely associated with improvement of antiretroviral treatment adherence. Of note,

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virologic failure followed 16% of low viremia episodes while genotype based optimization of antiretroviral regimen allowed to increase the rates of the complete virological suppression [7,10]. Risk factors for the incomplete virological suppression with low-level viremia include high pretreatment HIV-1 viral load (>6 log copies/ml), lower pretreatment lymphocyte CD4 cell count as well as treatment with lopinavir boosted with ritonavir [6].

Commonly used genotyping platform – Viroseq 2.8 genotyping system (Abbott Molecular, Abbott Park, IL) is intended for the use on samples with HIV viral loads >2000 copies/ml, however methodologies to improve efficacy of this assay below this threshold have been described [11]. Drug resistance testing is usually possible at viral loads >350–500 copies/ml and may be attempted at lower levels of viremia [1]. In this work we wish to present the efficacy and results of the bulk genotyping in samples with viral load <2000 copies/ml using combined Viroseq/in-house methodology.

2. Methods

All genotypic analyses were performed in a Clinical Laboratory at the Department of Infectious, Tropical Diseases and Immune Deficiency, Pomeranian Medical University, Szczecin, Poland. The protocol of the study was approved by the Bioethical committee of the Pomeranian Medical University, Szczecin, Poland, approval number KB-0012/08/12.

For this study, samples with genotyping test performed at plasma viremia levels <2000 copies/ml and >50 copies/ml were included (both antiretroviral naive and treatment failing patients). Majority of the treatment naive patients were viremic controllers. HIV RNA extraction, as well as reverse transcriptase and protease genotyping, were performed using a genotyping assay (Viroseq 2.8, Abbott Molecular, Abbott Park, IL, USA) according to manufacturer's protocol. If the Viroseq methodology based PCR signal was insufficient to perform the sequencing, a nested PCR was used with the following amplification primers: 5′-GCTCCTACTATGGGTTCTTTCTCTAACTGG (RT primer and reverse first round PCR primer), 5′-GAAGGGCACACAGCCAGAAATTGCAGGG (forward primer, first round PCR), 5′-TAGGAAAAAGGGCTGTTGGAAATGTGG (forward primer, second round PCR), 5′-CAAACTCCCACTCAGGAATCCA (reverse primer, second round PCR). The reverse transcription was performed at 42 °C for 45 min with Expand High Fidelity System (Roche Diagnostics, Mannheim, Germany). The PCR conditions were: denaturation for 2 min at 94 °C, primer annealing/elongation: 10 cycles of 15 s at 94 °C, 30 s at 55 °C, 1.5 min at 72 °C followed by 25 cycles of 15 s at 94 °C, 30 s at 55 °C, 1.5 min at 72 °C with addition of 5 s to the elongation time per cycle; final elongation of 7 min at 72 °C. The first round primers are based on the methodology published by Dilernia et al. [12]. This methodology and primer sequences were kindly provided by Prof. Richard Harrigan (HIV Centre of Excellence, Vancouver, Canada). Amplicons were used for sequencing on an ABI 3500 platform (Applied Biosystems, Foster City, CA) by standard techniques with BigDye technology. All reverse transcriptase and protease sequences were assembled with a Viroseq 2.8 software, if the assembly was not possible Recall online tool (http://pssm.cfenet.ubc.ca) was used. Both methods were quality-controlled by the external laboratory (QCMD) and with 99.4% concordance with control consensus sequence. Subtyping was performed using genotyping software (REGA genotyping 2.0 tool; http://bioafrica.mrc.ac.za/rega-genotype/html/subtypinghiv.html) based on the partial *pol* sequence obtained. Regardless the method, protease/reverse transcriptase sequences obtained were 1302 b.p. long; location from the start of HXB2 genome: at position 2253–3525. Drug resistance was evaluated based on the International AIDS Society-USA 2013 update [13].

3. Statistics

Continuous variables were analyzed using the Mann–Whitney *U*-test, while for nominal variables Fisher's exact test was used. Commercial software (Statistica 8.0PL, StatSoft, Warsaw, Poland) was implemented for statistical calculations.

4. Results

Analyzed group included 71 samples from 63 patients with HIV-1 plasma viremia <2000 copies/ml. 18 (18 patients) samples were from antiretroviral treatment naive and 53 (45 patients) from treated patients with virologic failure (plasma viremia >50 copies/ml). Plasma viremia ranged from 52 to 1980 copies/ml [median: 596 (IQR: 336–1244) copies/ml]. Among the treatment naive cases viremia ranged from 77–1933 copies [median 497 (IQR: 396–1301) copies/ml], while in treated patients viremia was from 52 to 1980 copies/ml [median: 621 (IQR: 277–1182) copies/ml]. Male subjects predominated in the group (44 cases, 69.8%). Heterosexual transmission route was the most common (26 cases, 41.3%), followed by injection drug users (20 patients, 31.7%) and men who have sex with men (17 cases, 17.0%). At genotyping, median age was 36 (IQR: 38–47) years for the treatment naive and 28 (IQR: 34–42) years for the treatment experienced patients. As expected, in the treatment naive group the CDC category A was notably more frequent (13 cases, 76.5%), then among experienced patients (15 cases, 32.6%) ($p = 0.01$). Symptomatic (CDC B category) infection and AIDS (CDC C category) were found in 3 (17.6%) and 1 (5.9%) naive patients versus 15 (32.6%), and 16 (34.8%) experienced ones, respectively.

In total, sequencing was successful in 62 (87.4%) samples with viral load <2000 copies/ml: in 31 (43.7%) cases using standard Viroseq system only, while in the remaining 31 (43.7%) samples Viroseq failed and in-house method was successful. For plasma viremia levels of 50–1000 copies the success rate was 39/45 (86.6%). In detail, success rates were as follows: 9/12 (75.0%) for viremia levels of 50–200 copies/ml, 19/21 (90.5%) for 201–500 copies/ml, 11/12 (91.67%) for 501–1000 copies/ml, and 23/26 (88.46%) for 1001–2000 copies/ml. As expected, plasma viremia among samples with successful Viroseq methodology genotyping were notably higher [range: 52–1933 copies/ml (median: 1054.5 (IQR: 406–1531)) copies/ml], compared to the samples successfully sequenced with the in-house method [range: 86–1164 copies/ml (median: 410.5 (IQR: 336–716))], $p = 0.008$.

Subtype B was identified in 45 (72.6%) cases. Among non-B variants, subtype D was the most common 10 (16.1%); three CRF02_AG (4.8%), two (3.2%) A1 and one (1.6%) CRF12_BF sequences were found. Frequency of identified non-B variants was not significantly different between Viroseq and in-house method [6 (19.4%) and 11 (35.5%), respectively].

Drug resistance mutations were found in 27 (43.5%) sequences, the overall resistance prevalence for NRTI was 30.65%, NNRI: 19.35%, PI: 11.29%. In samples from treatment naive patients 5/16 (31.25%) sequences with drug resistance mutations were found, among samples from antiretroviral treatment failing patients 24/46 (52.17%). In treatment experienced in two sequences both PI and NRTI mutations were found, in three NRTI/NNRTI and in two samples triple class NRTI/NNRI/PI resistance was observed. Frequency of drug resistance mutations is shown in Fig. 1. Drug resistance was also common (16/39, 41.0%) with viremia <1000 copies/ml. After stratification for different viremia ranges, detection of resistance was as follows: 50–200 copies/ml – in 5/9 (55.6%) sequences [3 NRTI, 3 NNRTI and 2 PI, including 1 double NRTI/NNRTI mutant and one triple class mutant], 201–500 copies/ml – in 7/19 (36.84%) sequences [5 NRTI, 2 NNRTI and 2 PI, including 1 double one triple class mutant] and 501–1000