Clinical impact of Ultra-Deep Versus Sanger Sequencing detection of minority mutations on the HIV-1 Drug Resistance interpretations after virological failure

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Aims

The study had 3 aims, comparison of Sanger sequencing and Ultra-deep sequencing (UDS); the comparison of the three most commonly used HIV drug resistance interpretation algorithms and the comparison of resistance interpretation of UDS data at two viral detection thresholds (1% and 20%).

Introduction

Drug resistance mutations are routinely detected using standard Sanger sequencing, which does not detect minor variants with a frequency below 20%. Several studies have clearly demonstrated that patients with mutation rates between 1% and 20%, particularly for NNRTIs, are more likely to fail therapy. It is therefore important to detect minor variants that occur below 20% frequency using a robust and sensitive method available at an affordable price and providing an easier interpretation for HIV drug resistance (DR) monitoring. The impact of detecting minor variants generated by UDS on HIV DR interpretations has not yet been studied.

Methods

Fifty HIV-1 patients who experienced virological failure were included in this retrospective study. The HIV-1 UDS protocol allowed the detection and quantification of HIV-1 protease and reverse transcriptase variants related to genotypes A, B, C, F and G. DeepChek®-HIV simplified DR interpretation software was used to compare Sanger sequencing and UDS.

Results

The total time required for the UDS protocol was found to be approximately three times longer than Sanger sequencing with equivalent reagent costs. UDS detected all of the mutations found by population sequencing and identified additional resistance variants in all patients. An analysis of DR revealed a total of 643 and 224 clinically relevant mutations by UDS and Sanger sequencing, respectively. A significant difference in the DR interpretations for 19 antiretroviral drugs was observed between the UDS and Sanger sequencing methods. Y181C and T215Y were the most frequent mutations associated with interpretation differences.

Conclusion

We have clearly shown that the numbers of mutations detected using UDS 1%, UDS 20% and Sanger sequencing are significantly different. When compared with the Sanger method, the UDS 1% data showed more minor and major discrepancies than the UDS 20% data. A combination of UDS and DeepChek® software for the interpretation of DR results would help clinicians provide suitable treatments. A cut-off of 1% allowed a better characterisation of the viral population by identifying additional resistance mutations and improving the DR interpretation.