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Random lopinavir concentrations predict resistance on lopinavir-based antiretroviral therapy

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ABSTRACT

Considering that most patients who experience virological failure (VF) on lopinavir-based antiretroviral therapy (ART) fail due to poor adherence rather than resistance, an objective adherence measure could limit costs by rationalising the use of genotype antiretroviral resistance testing (GART) in countries with access to third-line ART. A cross-sectional study was conducted in a resource-limited setting at two large clinics in Kwazulu-Natal, South Africa, in patients experiencing VF (HIV-RNA > 1000 copies/mL) on lopinavir-based ART who had undergone GART. Associations between major protease inhibitor (PI) resistance mutations and random plasma lopinavir concentrations were explored. A total of 134 patients, including 31 children, were included in the analysis. The prevalence of patients with major PI resistance mutations was 20.9% ($n = 28$). A random lopinavir concentration above the recommended minimum trough of 1 μg/mL [adjusted odds ratio (aOR) = 5.81, 95% confidence interval (CI) 2.04–16.50; $P = 0.001$] and male sex (aOR = 3.19, 95% CI 1.22–8.33; $P = 0.018$) were predictive of the presence of at least one major PI resistance mutation. Random lopinavir concentrations of <1 μg/mL had a negative predictive value of 91% for major PI resistance mutations. Random lopinavir concentrations are strongly associated with the presence of major PI resistance mutations. Access to costly GART in patients experiencing VF on second-line ART could be restricted to patients with lopinavir concentrations above the recommended minimum trough of 1 μg/mL or, in areas where GART is unavailable, could be used as a criterion to empirically switch to third-line ART.

1. Introduction

In a recent systematic review, rates of virological failure (VF) on second-line protease inhibitor (PI)-based antiretroviral therapy (ART) in resource-limited settings were as high as 38% after 3 years [1]. However, PI resistance was only detected in 18% of isolates from patients with VF [1]. The World Health Organization (WHO) has recommended that countries should develop strategies for third-line ART, which has recently been made available in some countries, including South Africa [2]. Considering that most patients on PI-based ART fail due to poor adherence rather than resistance [3,4], empirically switching patients to third-line ART would be a waste of resources and would not address the reason for VF in most patients. Genotype antiretroviral resistance testing (GART), both to confirm the presence of PI resistance and to guide the choice of an appropriate third-line regimen, is expensive (ca. US$300 per test) [5], and laboratory facilities in resource-limited settings are limited. Predicting the likelihood of PI resistance in patients experiencing VF on second-line ART would limit access to GART to those patients who are most likely to require third-line ART or, in areas where GART is unavailable, could be used as a criterion to empirically switch to third-line ART.

A simple objective adherence measure could improve the management of patients in VF on second-line ART by identifying which patients are likely failing due to resistance rather than poor adherence. Although vulnerable to the effect of 'white coat adherence' [6] and drug–drug interactions, lopinavir plasma concentrations are an objective adherence measure that could be performed on plasma submitted for viral load testing or GART. Several studies have demonstrated the utility of random plasma lopinavir concentrations to predict virological responses on lopinavir-based ART [7,8]. In a small previous pilot study, we described the utility of random plasma and hair lopinavir concentrations to predict the presence of VF and possibly major PI resistance mutations in patients on second-line ART [9]. Hair lopinavir concentrations are an attractive adherence measure as they reflect longer-term adherence, but there are very few

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laboratories able to perform the assay, obtaining hair samples requires training, and patients may refuse or not have head hair available (hair growth is slower in other areas of the body and the assays have only been tested on head hair). In this study, associations between random plasma lopinavir concentrations and the presence of major PI resistance mutations were investigated in patients with VF on lopinavir-based ART from two large ART clinics in Kwazulu-Natal, South Africa.

2. Materials and methods

2.1. Study population and setting

Participants were initially enrolled as part of the 'Protease Cleavage Site' (PCS) cohort between April 2009 and December 2013 from King Edward hospital (KEH) ART clinic and McCord Hospital ART clinic (also known as 'Sinikithemba') in Durban, South Africa. KEH ART clinic is funded by the South African Department of Health (DoH). 'Sinikithemba' was previously supported by the DoH and the President's Emergency Plan for AIDS Relief before the clinic's closure in June 2012. Patients identified with VF (HIV-RNA > 1000 copies/mL) after a minimum of 6 months of lopinavir-based ART were considered for enrolment in the PCS study, which included GART and adherence counselling. During the study period, the standard South African second-line regimen consisted of lopinavir boosted with ritonavir prescribed every 12 h together with two nucleoside/-tide reverse transcriptase inhibitors (NRTIs). As per WHO guidelines, second-line initiation occurred either for VF on first-line ART or for toxicity/intolerability of first-line drugs. Children aged <3 years or weighing <10 kg at the time of ART initiation were started on lopinavir (Kaletra® syrup) every 12 h with two NRTIs as first-line ART as per national guidelines during the study period [10]. Older children received lopinavir as Kaletra® tablets or syrup.

2.2. Study design

A cross-sectional study was performed including adults and children (defined as ≤18 years of age at time of GART) with VF on lopinavir-based ART for a minimum of 6 months in a resource-limited setting. Associations between random lopinavir concentrations and the presence of major PI resistance mutations were explored.

GART was performed at the Hasso-Plattner Laboratory, Nelson Mandela School of Medicine, University of Kwazulu-Natal (Durban, South Africa) using a ViroSeq® HIV-1 Genotyping System (Abbott Molecular Inc., Des Plaines, IL) or a validated in-house assay [11]. The genotype sequences were edited using Sequencher® DNA analysis software [12]. Patients were categorised as having major PI resistance if they had one or more of the following major resistance mutations defined by the Stanford HIV drug resistance database [13]: V32I; L33F; M46I/L; I47V/A; G48V/M; I50V; I54V/T/A/L/M; L76V; V82A/F/T/S; I84V; and L90M. The Stanford HIV drug resistance scoring system for lopinavir was used to categorise the lopinavir resistance profiles as susceptible or as low-, intermediate- or high-level resistance. Viral load testing was performed at the National Health Laboratory Service, Inkosi Albert Luthuli Central Hospital (Durban, South Africa) using NucliSENS easyQ® HIV-1 nucleic acid sequence-based amplification (bioMérieux, Marcy-l'Étoile, France) or Abbott RealTime HIV-1 (Abbott Molecular Inc.) assays.

Random lopinavir concentrations were performed on stored plasma samples remaining after GART at the clinical pharmacology laboratory of the University of Cape Town (Cape Town, South Africa). Lopinavir plasma concentrations were measured using a protein precipitation extraction procedure followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. An AB SCIEX 4000 mass spectrometer (AB SCIEX, Concord, ON, Canada) was operated at unit resolution in the multiple reaction monitoring mode, monitoring the precursor ions at m/z 629.5 and the product ions at m/z 120.2. A lopinavir-d8 stable isotope was used as an internal standard. The precursor ions of the internal standard were monitored at m/z 637.6 and the product ions at m/z 191.2. The assay was validated over the concentration range of 0.0195–20 μg/mL. The intrabatch and interbatch accuracy statistics of the lopinavir assay validation were between 95.0% and 96.4% and between 96.2% and 99.1%, respectively, at high, medium and low quality control concentrations. The coefficient of variation was <3.9%.

2.3. Statistical analysis

Stata v.13.0 (StataCorp LP, College Station, TX) was used to perform the statistical analysis. Multivariate logistic regression was performed to identify associations with the presence of at least one major PI resistance mutation. The following variables were included a priori in the multivariate model: age; sex; duration on lopinavir; viral load at the time of VF; and lopinavir concentration ≥1 μg/mL, which is the minimum recommended trough for lopinavir [14].

2.4. Ethics

This study was reviewed and approved by the Human Research Ethics Committee of the University of Cape Town and the Biomedical Research Ethics Committee of the University of Kwazulu-Natal. Informed consent was received from each participant, which included consent for storage of blood samples for further research related to human immunodeficiency virus (HIV), before enrolment into the PCS study.

3. Results and discussion

A total of 164 patients fulfilled the eligibility criteria for entry into the study; 20 were excluded as their GART could not amplify, 1 was excluded due to a contaminated GART and 9 had missing data. Thus, 134 patients, including 31 children, were included. Of the 31 children, 6 were below the age of 3 years and were therefore initiated on lopinavir as first-line ART. All children weighed >10 kg at the time of ART initiation. Patient characteristics at the time of VF of lopinavir-based ART are shown in Table 1. In total, 28 patients (20.9%), including 16 men, harboured major PI resistance mutations. Moreover, 19 patients (14.2%) demonstrated high-level PI resistance and 9 patients (6.7%) had reduced susceptibility to lopinavir. The most common NRTI backbone was zidovudine and didanosine (50.7%), which reflects the national ART treatment guidelines during the study period [15]. Table 2 illustrates the range of lopinavir concentrations in patients harbouring at least one major PI resistance mutation. The mean random lopinavir concentration in patients with at least one major PI resistance mutation was 9.13 μg/mL (standard deviation 7.52 μg/mL). A scatterplot of lopinavir concentrations versus the number of major PI resistance mutations is shown in Fig. 1. Supplementary Figs S1 and S2 illustrate the frequency of major PI mutations in patients with a random lopinavir concentration ≥1 μg/mL and <1 μg/mL respectively. Table 3 shows the factors associated with the presence of at least one major PI resistance mutation. On multivariate analysis, the strongest association with the presence of at least one major PI resistance mutation was a random lopinavir concentration above the recommended trough (≥1 μg/mL) [adjusted odds ratio (aOR) = 5.81, 95% confidence interval (CI) 2.04–16.50; $P = 0.001$], followed by male sex (aOR = 3.19, 95% CI 1.22–8.33; $P = 0.018$). There was an association of duration on lopinavir-based ART with PI resistance in the univariate analysis, but this association was not significant in the multivariate analysis. Table 4 describes the diagnostic utility of a random lopinavir

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