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van Zyl, Gert

Jennings, Lauren

Kellermann, Tracy

et al.

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Urine tenofovir monitoring predicts HIV viremia in patients treated with high genetic barrier regimens

Gert VAN ZYL[1,2], **Lauren JENNINGS**[3], **Tracy KELLERMANN**[4], **Zukisa NKANTSU**[3], **Dolphina COGILL**[3], **Marije VAN SCHALKWYK**[5], **Matthew SPINELLI**[6], **Eric DECLOEDT**[4], **Catherine ORRELL**[3], **Monica GANDHI**[6]

[1]Division of Medical Virology, Department Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

[2]National Health Laboratory Service, Tygerberg Business Unit, Cape Town South Africa

[3]Desmond Tutu HIV Centre, Institute of Infectious Disease and Molecular Medicine and Department of Medicine, University of Cape Town, South Africa

[4]Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

[5]Division of Infectious Diseases, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

[6]Division of HIV, Infectious Diseases, and Global Medicine at UCSF/San Francisco General Hospital, San Francisco, California, USA

Abstract

Objective: Access to VL measurements is constrained in resource-limited settings. A lateral flow urine tenofovir (TFV) rapid assay (UTRA) for patients whose regimens include TFV offers an affordable approach to frequent adherence monitoring.

Design: We conducted a cross-sectional study of patients to assess the utility of UTRA to predict virologic failure (VF), defined as a VL> 400 copies/mL.

Methods: We assessed urine TFV among 113 participants at increased risk of VF (who had previous VF on this regimen or had previously been ≥30 days out of care), comparing low genetic barrier efavirenz (EFV)-regimens (n=60) to dolutegravir (DTG)- or ritonavir-boosted PI (PI/r)-based high genetic barrier regimens (n=53). Dried blood spots (DBS) for TFV-diphosphate and plasma for TFV concentrations were collected, with drug resistance assessed if VF present.

Results: Among 113 participants, 17 of 53 received DTG or PI/r had VF at the cross-sectional visit, with 11 (64.7%) demonstrating an undetectable urine TFV; the negative predictive value (NPV) of undetectable UTRA for VF was 85% (34/40); none of 16 sequenced had dual class drug

Corresponding Author: Monica Gandhi, University of California San Francisco, Division of HIV, Infectious Diseases, and Global Medicine at UCSF/San Francisco General Hospital, San Francisco, California, USA; monica.gandhi@ucsf.edu.

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resistance. In those treated with EFV-regimens the sensitivity was lower, as only 1 (4.8%) of 21 with VF had an undetectable UTRA (p<0.001).

Conclusion: Urine tenofovir testing had a high negative predictive value for VF in patients treated with DTG or ritonavir-boosted PI regimens, where VF was largely explained by poor drug adherence. Frequent monitoring with inexpensive lateral flow urine TFV testing should be investigated prospectively in-between viral load visits to improve VL suppression on DTG-based first-line therapy in resource-limited settings.

Keywords

urine tenofovir; lateral flow assay; dolutegravir; tenofovir; virologic failure; high genetic barrier

Introduction

Successful antiretroviral treatment (ART) as measured by virologic suppression is essential for the health of people living with HIV (PLWH) and to prevent onward transmission [1]. However, routine viral load (VL) testing has limited availability in resource-limited settings (RLS), is costly and often not performed more than annually [2] which could result in long periods of missed unsuppressed viral loads with possible disease progression or transmission [3].

Additional adherence support for those identified at increased risk of virologic failure (VF) requires more affordable ways to monitor treatment adherence and improve treatment success. Urine tenofovir (TFV) testing offers a non-invasive method to identify PLWH with short-term poor adherence to pre-exposure prophylaxis (PrEP) or treatment regimens including tenofovir disoproxil fumarate (TDF) [4] and is now available as a point-of-care lateral flow urine tenofovir rapid assay (UTRA) [4]. In a differentiated care model, UTRA may allow frequent monitoring to identify VF and achieve better VL suppression between infrequent VL monitoring visits.

The World Health Organization (WHO) now recommends a fixed-dose combination regimen of TDF , lamivudine (3TC) and dolutegravir (DTG) - (TLD) as the preferred first-line ART regimen [5–7]. TLD results in fewer cases of virologic failure due to a higher genetic barrier to resistance, good tolerability and low prevalence of integrase strand transfer inhibitor (INSTI) resistance among treatment naïve individuals [8]. VF with first-line DTG-containing ART rarely occurs from drug resistance [9]. We have previously shown that viremia among PLWH treated with ritonavir-boosted protease inhibitor (PI/r)-based regimens is also largely attributable to poor adherence with drug resistance being rare [10]. Interventions aimed at improving adherence in patients receiving PI/r-based regimens have been successful in suppressing VL without requiring a regimen switch [11].

With a low genetic barrier to resistance regimen (efavirenz-based), we recently showed that a combination of a detectable urine TFV assay and an elevated VL is strongly predictive of drug resistance [12]. In contrast, among PLWH treated with high genetic barrier regimens, such as DTG- or PI/r-based ART, viremia is expected to be associated with low drug

exposure from inadequate adherence, who, in a rationalized approach, require adherence support.

Our study aim was therefore to assess the utility of UTRA in predicting concurrent VF and drug resistance in participants at high-risk for VF in South Africa receiving low genetic barrier EFV-based regimens or high genetic barrier (DTG or PI/r) regimens.

Methods

Participant enrolment

Study participants were recruited from the Gugulethu Community Health Centre (CHC) in Cape Town, South Africa, between September 2020 and December 2021. PLWH ≥18 years old were eligible to participate if on a TDF-containing regimen with an increased risk of experiencing virologic failure, as defined by either 1) one or more episodes of VF (>400copies/ml; corresponding to the South African threshold for adherence support) while on their current regimen or 2) ≥30 days of being out of care in the previous year confirmed by pharmacy refill collection data.

Study procedures involved a single cross-sectional visit for collection of demographic and disease data, including age, gender, World Health Organization (WHO) clinical stage, current ART and recent CD4 cell count. Blood samples (EDTA plasma) were drawn for plasma VL, and for TFV concentrations, with 50 microliters pipetted on Whatman™ 903 protein saver to generate dried blood spots (DBS) for tenofovir diphosphate (TFV-DP) quantification. Plasma was also reserved for HIV-1 drug resistance testing in case of VF. TFV in the urine was detected using the UTRA which takes 2–3 minutes to develop after a few drops of urine are dropped on the card. Self-reported adherence over the past 30 days was measured using a Likert scale[12].

Participants were categorized as those treated with EFV regimens (n=60) or high genetic barrier regimens (n=53), either PI/r or DTG-based. VF was defined as having a VL > 400 copies/mL vs ≤ 400 copies/mL (defined as suppressed). Sample size estimation was based on precision (+/− 16%) which required 38 VF cases at an expected sensitivity of 42%.

Laboratory methods

VL testing was performed with the Alinity m HIV-1 assay (Abbott Laboratories. Abbott Park, Illinois, U.S.A.) or COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (Roche Diagnostics, Basel, Switzerland).

HIV drug resistance testing was performed by Sanger Sequencing of the HIV *protease*, *reverse transcriptase* and i*ntegrase* genes using a previously published and validated in-house method[13,14] and the CDC-developed assay marketed by ThermoFisher[15]; mutations were scored with the Stanford HIV drug resistance database and reported back to providers.

TFV-DP testing in DBS was performed as previously described [12] and total TFV in plasma was quantified [16]. The range of quantification was 27 to 6924 fmol/3 mm punch for TFV-DP in DBS and 10 to 2000 ng/mL for TFV in plasma.

Statistics

Statistical analyses were performed using R version 4.1.2[17] . Wilcoxon rank sum tests compared continuous variables between groups; Fisher exact tests were used for categorical variables; and percentages compared with a two-sample proportion test; correlations were assessed with Spearman's rank order correlation.

Ethics

The study was approved by the University of Cape Town Human Research Ethics Committee (HREC_102/2020). All participants provided written informed consent.

Results:

We enrolled 113 participants of whom 60 and 53 received EFV- and DTG- or PI/r-based regimens, respectively (Table 1). Participants enrolled with the criterion of previous VF were more likely to have current VF than those who had been out of care. Although CD4 counts were not statistically different between participants with VF versus VL suppression, participants with suppression were more likely to be WHO Stage 1 and those with virological failure to be WHO stage 3 (Table 1).

EFV-treated patients

Of the 60 participants treated with an EFV-based regimen, 21 (35%) had current VF. Of these, only one had undetectable urine TFV (Table 1); 20 underwent successful sequencing, of whom 18 had dual class (major NRTI and NNRTI) resistance (supplementary table). TFV-DP and TFV plasma concentrations were available for 58 and 51 participants treated with EFV-based regimens, respectively; 9 did not have plasma results as 8 cases were recruited before dry ice was arranged for shipment and another sample was mislabeled. There was no difference in median (IQR) TFV-DP or TFV concentrations between participants with VF or suppression (Table 1). TFV-DP in DBS and TFV levels in plasma were only weakly correlated (Figure 1).

PI/r or DTG treated patients:

A similar proportion of the 53 patients treated with either DTG or PI/r vs EFV-based regimens (n=17, 35%) had VF (Table 1), but 11 of 17 on DTG or PI/r had undetectable urine TFV (sensitivity of 64.7%; 95% confidence interval (CI): 38.3%−85.8%); which was significantly higher than for EFV-based regimens ($p<0.001$). The UTRA test had a high specificity (94.4%; 95% CI: 81.3%−99.3%) for virologic suppression, with 34 of the 36 participants having detectable urine TFV (Table 1). Of the 13 with undetectable urine TFV, 11 had VF so the positive predictive value (PPV) of UTRA was 84.6% (95% CI: 57.8% −95.7%) and the negative predictive value (NPV) was 85.0% (95% CI:76.3%−97.2%). Of the 16 of 17 participants with VF who had successful viral sequencing, only 5 had major NRTI mutations and none had dual class resistance (supplementary table). TFV-DP concentrations were available for all 53 participants and plasma TFV for 52. In those with VF, there was significantly lower median (IQR) TFV-DP and TFV plasma concentrations than in those with virologic suppression ($p<0.0001$) (Table 1 and Supplementary Figure). All 11 participants with VF who had undetectable urine TFV also had undetectable plasma

TFV, evident of short-term poor adherence, and which had a relative good correlation with longer-term adherence as measured by TFV-DP in DBS; Spearman's rank order correlation (95% CI): 0.69 (0.52–0.81); p<0.0001 (Figure 1).

Discussion

Given that INSTI-based therapy is now first-line worldwide - but that frequent viral load testing is not always available - inexpensive ways to monitor adherence in-between viral loads are important. The UTRA had a higher sensitivity for detecting VF in patients on high genetic barrier regimens, identifying patients with poor adherence requiring support; and could be performed between infrequent VL tests to reinforce adherence as it is more affordable than VL monitoring, available at the point-of-care, non-invasive, and well-received by participants[18].

UTRA has previously been shown to have utility in predicting future HIV seroconversion in patients receiving TDF for PrEP [19,20]. This analysis now shows that UTRA is more sensitive to detect VF in participants treated with high genetic barrier regimens, in whom there is a stronger association between viremia and concurrent poor adherence than in participants treated with EFV-based regimens, who likely have drug resistance, when viremic, and improving adherence would therefore have a limited impact to suppress VL [12].

Our study purposefully selected participants with prior VF or treatment discontinuations who would benefit from adherence support. Even amongst our participants with current virologic suppression, median (IQR) TFV-DP concentrations were low compared to prior studies: 394 (283–642) fmol/punch in those on EFV-based regimens and 498 (242–717) fmol/punch in those on PI/r or DTG-based regimens. A previous study in the same community found that participants on EFV-based regimens with TFV-DP concentrations ≤ 400 fmol/punch had up to a 30 times higher risk of VF at visits one or two months later than those with TFV-DP concentrations of > 800 fmol/punch, and also showing that median TFV-DP concentrations may be lower in an African setting than reported in other settings [21].

Viremic patients treated with EFV-based regimens have a high risk of resistance [12,22–24]. Moreover, NNRTI pre-treatment drug resistance levels are above the WHO threshold of 10% [22,25,26]. WHO guidelines now recommend DTG-based regimens as first-line [5,6], given their high "forgiveness" for poor adherence[27]. High genetic barrier-and forgiving regimens may require a different approach to treatment success monitoring than EFV-based regimens, with an emphasis to identify patients with inadequate adherence rather than those at risk of drug resistance.

For participants on high genetic-barrier regimens, the UTRA test had a NPV of 85%, meaning that only 15% of cases with detectable urine TFV had VF, and a PPV of 84.6%, meaning that the majority of participants with undetectable urine TFV had concurrent VF, likely due to poor adherence.

Limitations of the study are its cross-sectional nature and the ability of the urine test to only detect tenofovir. However, the majority of current first-line regimens in resource-limited settings include TDF in a fixed-dose combination tablet. UTRA may perform better when employed longitudinally at multiple visits, detecting patients with variable adherence who might be at high risk of future VF. Also, as the study participants were at high risk of VF, the PPV and NPV should in future be assessed in other populations.

Conclusion:

Our paper shows the utility of tenofovir monitoring in urine for first-line antiretroviral therapy to trigger adherence interventions in the global setting. Prospective studies to investigate the use of UTRA on adherence reinforcement are ongoing and will inform WHO guidelines on treatment monitoring in real-world settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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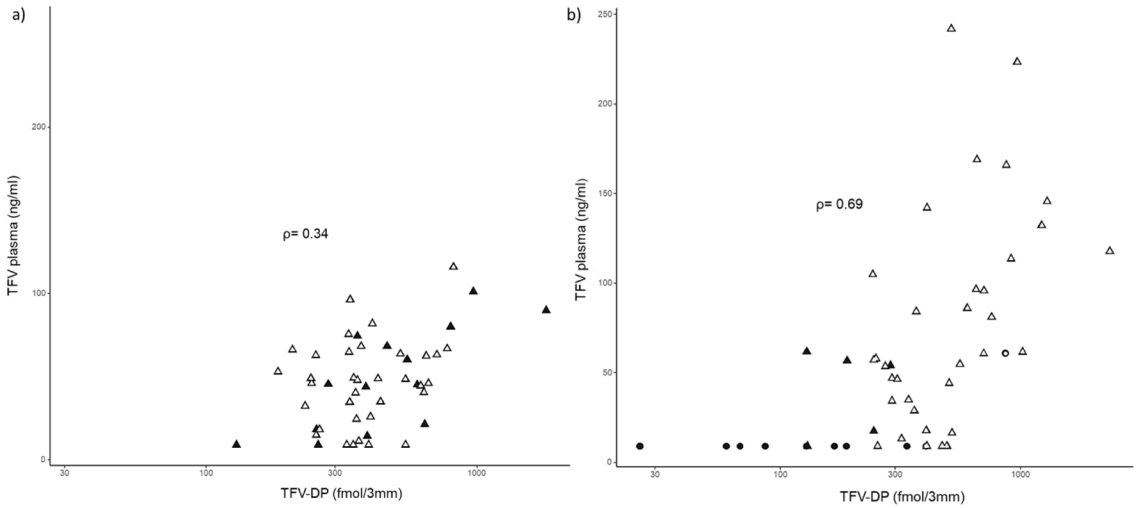

Figure 1: The association of UTRA results with long-term and short-term TFV exposure in participants treated with a) EFV- or b) PI/r- or DTG-based regimens.

DBS TFV- DP is plotted on X-axis (log scale) and total TFV in blood plasma on the Y-axis. Solid figures represent VF (VL > 400 copies/mL) and open figures represents VL suppression (VL ≤ 400 copies/mL) while round figures represent UTRA TN undetected and triangles UTRA TFV detected. Among EFV-treated participants, there was a weak positive Spearman's rank order correlation (95% CI): 0.34 (0.07–0.57); the one participant with undetectable TFV in urine had no TFV-plasma result, due to a sample labelling error, and is therefore not shown.

Among participants receiving PI/r or DTG-based regimens (panel b), those with VF and undetectable urine TFV (solid circles) most often had both low short term (TFV-plasma) and long-term (TFV-DP), whereas participants with suppressed VLs and detectable urine TFV (open triangles) had mostly higher long- and short-term TFV exposure, except for one outlying participant with undetectable urine TFV (open circle) despite high plasma TFV and DBS TFV-DP. There was a relatively good correlation between TFV-DP and plasma TFV; Spearman rank order correlation (95% CI) 0.69 (0.52–0.81).

Table 1:

Characteristics and Main Results by Virologic Failure

Characteristic	Current VL>400 (n=38)	Current VL≤400 (n=75)	Total (n=113)
WHO stage			
Stage 1	13 *	43 *	56
Stage 2	2	9	11
Stage 3	19 *	20 *	39
Stage 4	4	3	7
CD4 count median(IQR)	242(119–391)	281(97–484)	273(100–453)
Enrolment reason			
Return to care (RTC)	2 **	38 **	40
VL≥400	30 **	28 **	58
RTC &VL≥400	6	9	15
Regimen			
TDF/3TC/DTG	5	22	27
TDF/FTC/ATZ/r	4	3	7
TDF/FTC/EFV	21	39	60
TDF/FTC/LPV/r	8	11	19
Gender			
Female	25	51	76
Male	13	24	37
Age			
Median age (IQR)	39 (34–47)	38 (32–45)	38 (33–45)
TFV-exposure			
Urine tenofovir undetected			
EFV- regimens	1/21 (4.8%)	0/39 (0%)	60
PI/r or DTG regimens	11/17 (64.7%) **	2/36 (5.5%) **	53
TFV-DP in 3 mm DBS (fmol): median (IQR)			
EFV- regimens	360 (317–523)	394 (283–642)	365 (301–551)
PI/r or DTG regimens	128 (<27–188) **	498 (242–717) **	341 (189–599)
TFV plasma concentration (ng/mL) median (IQR)			
EFV- regimens	45 (19–73)	48 (32–65)	48 (25–67)
PI/r or DTG regimens	<10 (<10–11) **	59 (33–107) **	47 (<10–85)
Adherence			
Self-reported adherence % of 30 most recent days Median (IQR)	93(78–100)	97(90–100)	93(90–100)
Self-reported adherence score 2 6 point Likert scale: Median (IQR)	4(3–4) **	4(4–4.5) **	4(4–4)
Self-reported adherence score 3 Likert scale: Median (IQR)	4(3–5) **	5(4–5) **	5(4–5)

Characteristic	Current VL>400 (n=38)	Current VL≤400 (n=75)	Total (n=113)
Pharmacy refill adherence (%)	71(50–98) **	37(18–62) **	50(25–82)

* p<0.05

** p<0.01.

The lower limit of quantification for TFV-DP was <27 fmol/3 mm punch and < 10 ng/mL for plasma TFV. Self-reported adherence sore 2 was a 6-point Likert scale form worst (1) to best (6) adherence- responding to “In the last 30 days, how good a job did you do at taking your HIV medicines in the way that you were supposed to? “; and self-reported adherence score-2 was another 6-point Likert scale responding to “In the last 30 days how often did you take your HIV medicines in the way that you were supposed to?” scored from worst (1) to best (6).