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Title: Association between pharmacological tenofovir adherence measures and subsequent 24-week viral load outcomes for people with HIV in South Africa

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Abstract: 242/250 words

Background: Objective measures of antiretroviral therapy (ART) adherence, such as tenofovir (TFV) concentrations, may allow for targeted interventions to prevent future HIV viraemia. We evaluated three TFV adherence measures and their association with current and 24-week viral load (VL) outcomes.

Methods: In a South Africa-based trial, we measured urine TFV using a point-of-care (POC) antibody-based assay and two metrics assessed via liquid-chromatography-mass-spectrometry: quantitative urine TFV and TFV-diphosphate (TFV-DP) concentrations in dried blood spots (DBS). Logistic regression assessed the association between current and subsequent 24-week VL outcomes and these three measures. We also compared the baseline characteristics of individuals with detectable vs. undetectable POC urine TFV results at enrolment.

Results: Of 124 participants, 54.8% female, median age 39 years, 100 (81.5%) had detectable POC urine TFV and 23 (18.5%) did not. Higher TFV-DP concentrations in DBS were negatively associated with viraemia at 24-weeks (OR 0.83, 95% CI 0.725-0.928, p=0.003), whilst a detectable POC urine TFV (OR 0.62, 95% CI 0.22-1.82, p=0.380) and quantitative urine TFV (OR 0.98, 95% CI 0.95-1.00, p=0.153) showed no significant association. Compared to those with detectable POC urine TFV, those with undetectable POC urine TFV were more likely to be viraemic at enrolment (78.3% vs 25.7%, p<0.001) and have a CD4 count <200 cells/uL (34.8% vs 12.9%, p=0.001).

Conclusion: DBS TFV-DP was associated with viraemia at 24-weeks and could help predict future viraemia. Undetectable POC urine TFV was associated with recent ART initiation, current viraemia, and lower CD4 count.

Trial registration: Pan African Clinical Trials Registry (PACTR202001785886049) and the South African Clinical Trials Registry (DOH-27-072020-6890).

Introduction

Lifelong adherence to antiretroviral therapy (ART) is crucial for people with HIV (PWH), but assessing and supporting adherence can be challenging. Viral load (VL) measurement is the primary marker of treatment outcomes, with an undetectable VL <50 copies/mL indicating treatment success (1). However, VL monitoring is costly, cannot differentiate between poor adherence and viral resistance, and relies on centralized laboratories, with delayed results hindering timely interventions. In addition, measuring VL allows detection of established viraemia, which may leave little time to intervene before viraemia occurs, which can lead to clinical deterioration and transmission to partners. Measuring ART drug concentrations can objectively assess adherence and identify PWH at risk of future treatment failure despite current viral suppression, enabling early intervention before viral suppression is lost.

In South Africa, tenofovir disoproxil fumarate (TDF) continues to be widely used, with current (2023) HIV treatment guidelines recommending a fixed combination of TDF, lamivudine and dolutegravir (TLD) for first-line ART (2). Dolutegravir is well tolerated, effective in achieving and maintaining viral suppression, and has a high genetic barrier to resistance (3-4). The fixed dose combination of TLD means that measures of tenofovir (TFV) concentrations may be particularly useful for measuring overall adherence to this widely used single tablet combination.

Both short- and long-term measures of TFV adherence exist. Tenofovir-diphosphate (TFV-DP) concentrations measured in dried blood spots (DBS) by liquid chromatography-tandem

mass spectrometry (LC-MS/MS) are associated with TDF exposure in the preceding six to eight weeks (5), and can predict future viraemia, however this technology still requires samples to be sent to and processed in laboratories (1-3). More clinically accessible methods of TFV assessment are needed, such as antibody-based point-of-care (POC) urine TFV assays which provide a measure of recent TDF exposure (preceding three to four days), giving insight into recent or short-term adherence (9). POC-TFV assays can provide results within minutes in a clinic setting. Use of POC TFV assays in combination with adherence counselling has already been associated with high levels of resuppression after virological failure (10). Furthermore, POC-TFV, quantitative urine TFV by LC-MS/MS and TFV-DP are associated with concurrent viraemia (11-12). However, whether POC-TFV and quantitative urine TFV measures are associated with future viraemia has not been demonstrated.

This study aimed to determine whether TFV-DP measurement on DBS, quantitative urine TFV measured in the laboratory, and POC-TFV, are associated with subsequent 24-week VL values. It also aimed to compare the baseline characteristics of individuals in whom TFV was and was not detected in the urine (based on the POC-TFV assay).

Methods

Study design

We conducted a sub-study within the POwER trial, a feasibility study comparing POC VL testing with standard laboratory VL testing among people with recent viraemia (VL ≥1000 copies/mL), which has been described previously (13,14).

Setting

The study took place at an urban and rural clinic in KwaZulu-Natal, South Africa.

Participants

We included all POwER participants who were taking TDF. PWH were eligible for POwER if they were receiving first-line dolutegravir or efavirenz-based ART and with recent viraemia >1000 copies/mL in the past 6 weeks, for which they had not yet received enhanced adherence counselling (EAC) as recommended by the World Health Organization (WHO).

Procedures

Consent for this sub-analysis was included in the original POwER consent. Baseline demographic, behavioural, and clinical characteristics of participants were collected at enrolment through nurse or counsellor-conducted questionnaires. Enhanced adherence counselling recommended by the South African National Department of Health HIV guidelines was conducted for all participants, and all consenting participants had urine, whole blood and plasma samples taken and stored at -80°C, for retrospective testing. Participants were randomized to receive either POC or standard laboratory-based repeat VL testing after 12 weeks, with the primary outcome VL measured 24-weeks after enrolment (Supplement Table S1). The VL measured at enrolment is considered current VL in this study.

Laboratory testing

Urine was thawed and sent for quantitative urine TFV adherence testing using LC-MS/MS (Supplement Section 1:Liquid chromatography and dual tandem mass spectrometry (LC-MS/MS) sample analysis protocol) at the Africa Health Research Institute (AHRI) in Durban, and for POC-TFV assay (Abbott Laboratories, Abbott Park, IL, USA) testing at the CAPRISA Clinical Trial Unit Laboratory. The Abbot POC-TFV assay is an antibody-based test for TFV that utilizes Lateral Flow Immunoassay (LFA) technology and was previously validated against the reference standard of TFV detection and quantification, LC-MS/MS (4). The POC-TFV assay has a threshold (concentration of ≥1500 ng/ml) to determine whether a person has taken at least one dose of TDF within the prior four days. Specifically, 3-4 urine drops were added to the test well, with the result read by two independent laboratory technicians after 3-5 minutes. Photos of discrepant results were adjudicated by a third investigator. For TFV-DP measurement, DBS were prepared from EDTA specimens and stored at -80°C before being thawed and analysed using LC-MS/MS at AHRI. 24-week VL was measured in thawed plasma samples at the National Health Laboratory Service at the Inkosi Albert Luthuli Hospital in Durban (Supplement Section 2: Details of HIV viral load testing). Urine TFV and DBS TFV-DP are stable when stored at -80 degrees centigrade and the POC-TFV and TFV-DP assays have been validated on frozen samples. (5, 6)

Exposures and outcomes

For the primary analysis, the exposures of interest were POC-TFV, TFV-DP in DBS, and quantitative urine TFV using LC-MS/MS, with the primary outcome being viraemia (VL>50 copies/mL) at 24-weeks.

In a secondary analysis to describe baseline characteristics associated with a negative POC-TFV result, the exposures of interest were baseline sociodemographic and laboratory (including current VL and CD4 cell count) factors, and the outcome of interest was detectable vs. undetectable urine POC-TFV.

Statistical analysis

For the main analysis, we used separate univariable logistic regression models to analyze the relationship between each of the measures of adherence (binary POC-TFV [detected versus not-detected], quantitative urine TFV and quantitative TFV-DP in DBS as independent exposures) and the outcome of 24-week viraemia (VL > 50 copies/ml). We rescaled TFV-DP to every increase of 100 fmol/punch, and urine TFV to every increase of 1000 ng/mL, to aid in interpretation of odds ratios. In a sensitivity analysis, we included an interaction term between the TFV measures and a binary exposure variable of baseline ART regimen (dolutegravir or efavirenz-based), to evaluate whether any associations differed by ART regimen. In a post-hoc sub-analysis restricted to people with baseline undetectable VL <50 copies/mL, we determined the number of people who have "TFV-DP/VL mismatch" i.e. undetectable VL but a low TFV-DP <800 fmol/punch (threshold defined based on findings from previous studies(1, 7)), and described the proportion with 24-week viraemia, and with undetectable POC urine TFV, in those with and without mismatch.

In a secondary analysis, we described baseline clinical and laboratory characteristics of participants with detectable versus undetectable TFV in the urine, using the POC TFV assay, and compared the proportions using chi-squared tests.

Ethical approval

The POwER study received approval from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC00000836/2019) and the University of Oxford Tropical Research Ethics Committee (OxTREC66-19).

Results

Study Population

A total of 124 participants who were taking TDF were enrolled into the POwER study between 20 August 2020 and 25 March 2022. The majority were women (68/124, 54.8%) and the median age was 39.0 years (interquartile range (IQR) 34.0 to 45.0 years, Table 1).

Participants had been on ART a median duration of 4.1 years (IQR 1.5 to 6.1 years) and had been on their current regimen (40.3% dolutegravir -, 59.7% efavirenz-based regimen) for a median of 1.6 years (IQR 0.7 to 5.0 years), all had been on TDF-containing ART for ≥3 months prior to enrolment. Participants presented a median of 15 days (IQR 12.8 to 21.0 days) since their pre-enrolment detectable VL result, that determined their eligibility for POwER.

During the initial phase of enrolment into the POwER study, we were informed that 45 participants had their pre-enrolment VLs measured on a defective VL analyzer which overestimated some VL results meaning that a higher proportion of enrolled participants were virally suppressed. None of the affected VLs were from timepoints used in this analysis. After being informed, all the affected participants were agreeable to continue in POwER and were included in this sub-study in order to have participants with a history of both viraemia and viral suppression.

Of the 124 participants included in the current analysis, 100 (81.5%) had detectable TFV in their urine, using the POC-TFV assay, and 23 (18.5%) did not (**Table 1**). Overall, the median quantitative urine TFV concentration was 20000 ng/mL (IQR 7280 to 33625) and median TFV-DP concentration was 734.2 fmol/sample (IQR 471.0 to 1015.2).

Association between TFV and TFV-DP measures and subsequent 24-week VL outcomes.

Of the 124 participants, one participant did not have a 24-week VL, despite attending the 24-week visit, and was excluded from the analysis. Four (3.2%) were lost to follow-up (missed week 24 visit) and were assumed to have an unsuppressed 24-week VL.

We did not find evidence that the POC-TFV assay (odds ratio [OR] 0.622, 95% CI 0.222-1.920, p=0.380, Table 2 and Supplement Table S2) or quantitative urine TFV concentrations (OR 0.982, 95% CI 0.954-1.000, p=0.153) were associated with 24-week viraemia. In contrast, DBS TFV-DP concentrations were strongly associated with 24-week viraemia (OR 0.828, 95% CI 0.725-0.928, p=0.003), meaning for every 100 fmol/punch increase in TFV-DP the odds of viraemia decreased by 0.83. Participants with viraemia at 24 weeks had a median baseline DBS TFV-DP of 500 fmol/punch, versus 750 fmol/punch in those who were virally suppressed (Supplement Figure S1).Results did not change in sensitivity analyses excluding people who were lost to follow-up (Supplement Table S3). When restricting to people with undetectable VL at enrolment (n=57), 18 (31.6%) had ‘mismatch’ with a low TFV-DP <800 fmol/punch. 2/18 (11.1%) of those with mismatch had an unsuppressed 24-

week VL, versus 0/39 (0.0%) without mismatch. One person with mismatch (5.6%) and one person without mismatch (2.6%) had undetectable POC urine TFV (Table 1).

The effect of baseline ART regimen on the association between TFV and TFV-DP measures and subsequent 24-week VL outcomes.

In the sensitivity analysis testing for effect modification by baseline ART regimen (dolutegravir - versus efavirenz-based ART) (**Table 2)** there was no evidence that the baseline ART regimen modified the association between the subsequent 24-week VL outcomes and POC-TFV (likelihood ratio test for interaction [LRT] p=0.477), quantitative urineTFV (LRT p=0.362) or DBS TFV-DP (LRT p=0.854).

Characteristics of study participants stratified by POC urine TFV results

There was no significant difference between people with detectable and undetectable POC-TFV results based on age, sex, ethnicity, ART regimens or the presence of reported ART side effects. Compared to participants with detectable TFV, those with undetectable TFV, had been on ART a median of 2.0 (IQR 0.7-4.4) years versus 4.3 (IQR 2.1-6.2) years (p=0.002) and on their current regimen a median of 0.8 (IQR 0.5-2.1) years versus 2.1 (IQR 0.7- 5.0) years (p=0.017). At enrolment, a greater proportion of participants with undetectable TFV had a CD4 count <200 cells/uL compared to those with detectable TFV (34.8% vs 12.9% p=0.001) and a higher proportion had current viraemia (VL ≥1000 copies/mL) (78.3% vs 25.7% p<0.001).

Discussion

This analysis examined three ART adherence measures and their association with subsequent 24-week VL outcomes. Only TFV-DP concentrations in DBS showed a statistically significant association with future viraemia. While the odds ratios for POC-TFV and quantitative urine TFV for future viremia were less than one, the confidence intervals were broad and included one. We also found that those with undetectable TFV were more likely to be earlier in their ART journey, have lower CD4 counts, and have current viraemia.

TFV-DP in DBS has been shown to correlate with HIV treatment outcomes but requires centralized laboratory processing (5-8,16-17,), while POC-TFV assays may be used in clinics. Although other studies have shown the relationship between POC-TFV and current

viremia, ours is the only study, to our knowledge, to evaluate the relationship between POC-TFV results and future VL outcomes. We also found that when restricted to people with undetectable VL at baseline, people with TFV-DP/VL mismatch had a higher proportion of subsequent 24-week viraemia than those without mismatch, although numbers were too small for formal comparison. Further work is required to determine whether TFV-DP could be used to identify people with current viral suppression, in whom an adherence intervention based on pharmacologic measures could prevent future viremia.

In our study, we found no association between POC-TFV and quantitative urine TFV results and subsequent 24-week viraemia. This may be due to our relatively small sample size, however the lack of association between urinary TFV (POC) and viremia most likely reflects its short detection window (hours-days), unlike TFV-DP in DBS which integrates longer-term adherence over weeks and shows stronger virologic correlations.
. We previously showed that POC-TFV results were associated with concurrent viraemia measured at the same timepoint (11). Therefore, the POC-TFV assay, which has been shown to be acceptable in pre-exposure prophylaxis studies (18) and a recent qualitative study in Cape Town (19), may be useful in public primary healthcare clinics for rapidly detecting poor adherence and guiding counselling for PWH to avert negative outcomes. In addition, adherence interventions with feedback using the POC-TFV assay are associated with improvements in virologic suppression and are being more widely studied (10). Implementation studies and randomized controlled trials are underway to evaluate the use of POC-TFV assays among PWH in South Africa, and the updated South African ART guidelines now make provision for detection of antiretroviral drugs in blood or urine to assess adherence, which could assist with targeting HIV drug resistance testing or directing individuals into differentiated models of care (2).

Furthermore, our finding that people with a negative POC-TFV assay result were more likely to be at an earlier stage of ART, have lower CD4 cell counts, and are more likely to have current viraemia suggesting that early timepoints are critical for PWH, and more focus should be placed on providing early adherence support after ART initiation. In addition to baseline CD4 count in other studies, age and sex are important predictors of future treatment failure (8, 20).

This study's strengths lie in its comparative analysis of three different pharmacological measures of TFV adherence and its focus on future viraemia as opposed to current viraemia.

The study took place in South Africa, a high disease burden setting, which would benefit from timely cost-effective strategies to assess and manage adherence.

Limitations include a small sample size and restricted geographical scope, with data collected from only two sites. The study also had a relatively short follow-up period. Consequently, larger, longer-term studies are warranted to validate these findings and provide more comprehensive insights.

Measuring TFV-DP in DBS is strongly associated with subsequent 24-week viraemia, in both people receiving dolutegravir- or efavirenz-based ART and could provide an opportunity to target adherence interventions, reduce viraemia and prevent drug resistance. The association of undetectable POC-TFV with current viraemia and lower CD4 counts earlier after ART initiation suggests future interventions to avert poor outcomes.

Supplement Testing_NB[35]-http://links.lww.com/QAI/C668

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Table 1: Baseline characteristics of participants stratified by urine TFV results

Characteristic	Variable	Total	TFV Not Present (n=23)	TFV Present (n=101)	p-value
Age, years	Median (IQR)	39.0 (34.0 to 45.0)	38.0 (34.0 to 51.0)	39.0 (34.0 to 45.0)	0.938
Gender	Female	68 (54.8)	9 (39.1)	59 (58.4)	0.108
	Male	56 (45.2)	14 (60.9)	42 (41.6)	
Time since ART initiation, years	Median (IQR)	4.1 (1.5 to 6.1)	2.0 (0.7 to 4.4)	4.3 (2.1 to 6.2)	0.002
Time on current regimen, years	Median (IQR)	1.6 (0.7 to 5.0)	0.8 (0.5 to 2.1)	2.1 (0.8 to 5.2)	0.017
Time on dolutegravir-based regimen, years	Median (IQR)	0.6 (0.5 to 1.0)	0.6 (0.5 to 1.1)	0.6 (0.5 to 1.0)	0.807
Current TB	No	122 (98.4)	23 (100.0)	99 (98.0)	1.000
	Yes	2 (1.6)		2 (2.0)	
ART side effects	No	120 (96.8)	23 (100.0)	97 (96.0)	1.000
	Yes	4 (3.2)		4 (4.0)	
Last time participant missed a dose of ART	<2 weeks	30 (24.2)	11 (47.8)	19 (18.8)	0.014
	2-4 weeks	16 (12.9)	3 (13.0)	13 (12.9)	
	1-3 months	16 (12.9)	4 (17.4)	12 (11.9)	
	>3 months	8 (6.5)	1 (4.3)	7 (6.9)	
	Never	54 (43.5)	4 (17.4)	50 (49.5)	
Number of ART doses missed in past 4 days	0	95 (76.6)	11 (47.8)	84 (83.2)	<0.001
	1	14 (11.3)	3 (13.0)	11 (10.9)	
	2	9 (7.3)	5 (21.7)	4 (4.0)	
	3	2 (1.6)	1 (4.3)	1 (1.0)	
	4	4 (3.2)	3 (13.0)	1 (1.0)	
Ethnicity	Black African	121 (97.6)	22 (95.7)	99 (98.0)	0.463
	Coloured	1 (0.8)		1 (1.0)	
	Other	2 (1.6)	1 (4.3)	1 (1.0)	
Enrolment CD4 count category, cells/μL	<200	21 (16.9)	8 (34.8)	13 (12.9)	0.001
	200-349	25 (20.2)	7 (30.4)	18 (17.8)	
	350-499	29 (23.4)	6 (26.1)	23 (22.8)	
	>=500	49 (39.5)	2 (8.7)	47 (46.5)	
Time since pre-enrolment viral load, days	Median (IQR)	15.0 (12.8 to 21.0)	15.0 (13.5 to 18.0)	14.0 (12.0 to 21.0)	0.747
ART regimen at enrollment	TDF / 3TC / DTG	49 (39.5)	12 (52.2)	37 (36.6)	0.237
	TDF / FTC / EFV	75 (60.5)	11 (47.8)	64 (63.4)	
Urine tenofovir concentration, ng/mL	Median (IQR)	20000 (7280 to 33625)	207 (0.0 to 987)	23100 (14400 to 37100)	<0.001
>1500 ng/mL			4 (3.9%)	99 (96.1%)	
≤1500 ng/mL			20 (95.2%)	1 (4.8%)	
DBS tenofovir diphosphate	Median	734.2 (471.0 to	263.4 (40.2 to	814.7 (576.4 to	<0.001

Characteristic	Variable	Total	TFV Not Present (n=23)	TFV Present (n=101)	p-value
concentration, fmol/sample	(IQR)	1015.2)	429.9)	1162.5)	
>800 fmol/punch			1 (2.6%)	38 (97.4%)	
≤800 fmol/punch			1 (5.6%)	17 (94.4%)	
Enrolment viral load	<50 copies/mL	57 (46.0)	2 (8.7)	55 (54.5)	<0.001
	50-999 copies/mL	23 (18.5)	3 (13.0)	20 (19.8)	
	≥1000 copies/mL	44 (35.5)	18 (78.3)	26 (25.7)	

Table 2: Logistic regression models of the association between point-of-care urine tenofovir results, quantitative urine tenofovir concentrations, and dried blood spot tenofovir diphosphate concentrations, and subsequent 24-month viraemia

	Overall		Dolutegravir-based ART		Efavirenz-based ART	
Logistic regression models						
	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P
Viraemia ≥50 copies/mL						
Positive point-of-care urine TFV test	0.62 (0.22-1.92)	0.380	0.96 (0.23-5.04)*	0.962	0.44 (0.10-2.31)*	0.290
Urine TFV concentration (increase of 1000 ng/mL)	0.98 (0.95-1.00)	0.153	0.97 (0.93-1.00)†	0.121	0.99 (0.96-10.3)†	0.749
DBS TFV-DP concentration (increase of 100 fmol/punch)	0.83 (0.73-0.93)	0.003	0.840 (0.68-1.00)‡	0.079	0.82 (0.68-0.96)‡	0.024

* LRT for interaction p = 0.477, †LRT for interaction p = 0.362, ‡LRT for interaction p = 0.854