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BRIEF REPORT

Urine Tenofovir Levels Strongly Correlate With Virologic Suppression in Patients With Human Immunodeficiency Virus on Tenofovir Alafenamide-Based Antiretroviral Therapy

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We found that urine tenofovir (TFV) levels >1500 ng/mL strongly predict virologic suppression among people with human immunodeficiency virus taking tenofovir alafenamide (odds ratio, 5.66; 95% confidence interval, 1.59–20.14; $P = .007$). This suggests an existing point-of-care assay developed for tenofovir disoproxil fumarate will support adherence monitoring for patients on all TFV-based antiretrovirals.

Keywords. tenofovir alafenamide; adherence; viral suppression; point-of-care monitoring.

For people with HIV, optimal adherence, defined as taking antiretroviral therapy (ART) 80% to ≥95% of the time depending on regimen [1] is considered a requirement to reduce morbidity/mortality and avoid inflammation that may occur with suboptimal adherence [2]. Treatment-as-prevention, the principle that virologically suppressed patients cannot transmit HIV, also relies heavily on adherence.

To support objective adherence monitoring and interventions, we previously developed a highly sensitive/specific immunoassay to measure urine tenofovir (TFV) levels among patients taking tenofovir disoproxil fumarate (TDF) [3, 4]. This immunoassay has been developed into a point-of-care (POC) lateral flow assay that accurately predicts recent TDF ingestion, providing a real-time yes/no answer as to whether a patient has taken TDF within the past 5 days, using a urine TFV cutoff of 1500 ng/mL.

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Both TDF and tenofovir alafenamide (TAF) are commonly used for HIV treatment and prevention. Unlike TDF (which is metabolized in the gut/plasma), TAF is metabolized intracellularly, resulting in 75% lower urine TFV levels [5]. Consequently, it was previously thought that, although a urine assay will be useful to monitor adherence on TAF [6], a separate urine TFV cutoff (proposed at 300 ng/mL based on modeling [7]) may be needed [5].

Yet because a TAF-specific urine assay does not yet exist, and would require additional time, resources, and funding to develop, we worked to determine whether the existing immunoassay developed for TDF could have real-world applicability among patients taking TAF. We thus explored whether urine TFV levels above the 1500 ng/mL cutoff established for TDF correlate with virologic suppression (VS) among people with HIV taking TAF.

METHODS

We collected urine samples at 2 San Francisco HIV clinics from June 2019 through December 2021. Participants were recruited by fliers and met with study coordinators to determine eligibility, complete an adherence questionnaire, and provide urine samples.

Eligible participants were required to be 18+ years old, taking TAF, and able to: (1) give informed consent, (2) provide ≥1 oz of urine, and (3) provide no more than 1 sample every 30 days. To enrich our sample for individuals with adherence challenges, participants from POP-UP (a clinic serving patients with homelessness/unstable housing [8], embedded within 1 of our sites) received proactive outreach. Because we previously demonstrated that individuals have similar urine TFV concentrations on TAF whether dosed at 25 mg or 10 mg (with pharmacologic boosters) [9], we did not exclude participants based on TAF formulation. Urine TFV levels were measured by liquid chromatography tandem mass spectrometry at the University of California San Francisco (UCSF) using methods validated for urine [3, 4].

We used electronic medical records to collect information on each participant's ART as well as characteristics (assessed at/around the time of urine collection) including age, sex, gender, weight, creatinine, and glomerular filtration rate (GFR). For each participant, we recorded the HIV RNA obtained at or most recently after the urine collection visit. In cases in which a participant provided more than 1 urine sample before an HIV RNA was checked, we included the urine sample collected most recently before the RNA. Individuals could be included more than once, for separate patient visits, if they submitted separate urine samples associated with different RNA checks.

Table 1. Baseline Participant Characteristics and Urine TFV Levels Among Urine Samples Associated With and Without Virologic Suppression, Collected From People With HIV on TAF at 2 San Francisco HIV Primary Care Clinics, June 2019–December 2021

Variable	Viral Load >200 Copies/mL (n = 15 Samples)	Viral Load <200 Copies/mL (n = 68 Samples)	*P* Value
Age (y), median (IQR)	45 (38, 59)	55 (39, 60.5)	.49
Current gender identity	…	…	.23
Cis-female	0 (0%)	7 (10%)	…
Trans-male	0 (0%)	1 (1%)	…
Cis-male	10 (67%)	51 (75%)	…
Trans-female	4 (27%)	8 (12%)	…
Nonbinary	1 (7%)	1 (1%)	…
Sex assigned at birth	…	…	.16
Female	0 (0%)	8 (12%)	…
Male	15 (100%)	60 (88%)	…
Race/ethnicity	…	…	.18
White	5 (33%)	25 (37%)	…
Black	5 (33%)	31 (46%)	…
Latinx	4 (27%)	5 (7%)	…
Other/missing	1 (7%)	7 (10%)	…
Weight (kg), median (IQR)	73.7 (62.6, 89.7)	81.6 (69.9, 96.6)	.20
Creatinine (mg/dL), median (IQR)	0.85 (0.73, 0.98)	1.02 (0.90, 1.22)	.052
GFR (mL/min)	…	…	1.00
<60	2 (13%)	9 (13%)	…
60+	13 (87%)	57 (84%)	…
Borderline	0 (0%)	2 (3%)	…
Time since ART (h), median (IQR)	17 (5.6, 64.6)	7.3 (5.3, 15.1)	.23
ART doses missed in past 7 d, median (IQR)	2 (0, 4)	0 (0, 0.5)	<.001
Percent of time ART taken (self-report), median (IQR)	90 (70, 100)	100 (92.5, 100)	.04
TAF formulation	…	…	.19
25 mg (unboosted)	6 (40%)	37 (54%)	…
10 mg (boosted)	7 (47%)	29 (43%)	…
25 mg (boosted)	2 (13%)	2 (3%)	…
Median number of d between urine collection and viral load (IQR)	6 (0, 17)	63 (13, 119)	.006
Liquid chromatography tandem mass spectrometry results (urine TFV level, μg/mL), median (IQR)	0.69 (0.07, 2.10)	3.19 (1.46, 5.72)	<.001
Log transformed urine TFV concentrations, median (IQR)	−0.37 (−2.65, 0.74)	1.16 (0.37, 1.74)	<.001

Abbreviations: ART, antiretroviral therapy; GFR, glomerular filtration rate; IQR, interquartile range; TAF, tenofovir alafenamide; TFV, tenofovir.

We used generalized estimating equations to fit models comparing urine TFV levels (above/below 1500 ng/mL) for urine samples with and without VS (HIV RNA <200 copies/mL). We also compared baseline characteristics of samples provided from suppressed versus unsuppressed patients (eg, suppressed/unsuppressed samples), using Chi squared or Fisher exact testing for categorical variables and Wilcoxon rank-sum testing for continuous variables. All statistical analyses used STATA 17.0. The UCSF institutional review board approved this study.

RESULTS

Our analysis included 83 samples (68 suppressed, 15 unsuppressed) from 67 unique individuals. Samples from person-visits with and without VS were similar in: age (median 55 vs 45 years), sex (88% vs 100% male), gender (75% vs 67% cis-male), race/ethnicity (46% vs 33% Black; 37% vs 33% White), GFR (84% vs 87% ≥ 60 mL/min), median creatinine (1.02 vs 0.85 mg/dL), weight (81.6 vs 73.7 kg), and TAF formulation (54% vs 40% on unboosted 25 mg); all with $P > .05$ (Table 1).

Comparing suppressed with unsuppressed samples (Table 1), median times since last self-reported ART were 7.3 versus 17 hours ($P = .23$). The self-reported percentages of time that ART was taken were 100% versus 90% ($P = .04$) and the median number of ART doses missed in the last 7 days was 0 versus 2 ($P < .001$). Median times between urine collection and RNA measurement were longer in suppressed samples (63 vs 6 days; $P = .006$), likely because of more frequent RNA checks among patients considered at risk for viremia. Median urine TFV levels by liquid chromatography tandem mass spectrometry were 3.19 μg/mL (3190 ng/mL) for suppressed and 0.69 μg/mL (690 ng/mL) for unsuppressed samples.

In unadjusted modeling, urine TFV levels >1500 ng/mL were strongly associated with VS (odds ratio [OR], 6.00; 95%

confidence interval [CI], 1.73–20.75; $P = .005$). This finding was similar in an adjusted model (Supplementary Table 1) incorporating age, race/ethnicity, and GFR (OR, 5.66; 95% CI, 1.59–20.14; $P = .007$). Sex was not included in adjusted modeling (because no unsuppressed samples were collected from females). We also performed a sensitivity analysis using a urine TFV cutoff of 300 ng/mL, which correlated with VS albeit to a lesser extent (adjusted OR, 4.21; 95% CI, 1.29–13.69; $P = .017$). When incorporating both self-reported adherence and the 1500 ng/mL TFV cutoff into the model, only the TFV cutoff retained significance (adjusted OR, 4.80; 95% CI, 1.23–18.81; $P = .02$) versus missed doses in the past week (adjusted OR, 0.92; 95% CI, .74–1.15; $P = .48$).

The sensitivity, specificity, positive predictive value, and negative predictive value of the 1500 ng/mL cutoff for VS on TAF were 75.0%, 66.7%, 91.1%, and 63%, respectively.

DISCUSSION

Urine TFV levels >1500 ng/mL were strongly predictive of VS (defined as VS on the RNA obtained on or most recently after urine sample collection) among people with HIV on TAF. This finding suggests that the POC immunoassay developed for TDF could have real-world applicability among patients taking TAF for HIV treatment or prevention.

A separate POC urine assay was thought previously to be needed for TAF because of the lower plasma/urine TFV concentrations seen with TAF compared with TDF [5]. Based on modeling from directly observed therapy studies, our team has proposed a cutoff of 300 ng/mL to maximize sensitivity for nonadherence out to 5 days post-TAF ingestion [7]. Yet, our results now show, in a real-world setting, likely because of improved specificity, that the odds of VS are more than 5 times higher among people on TAF with urine TFV levels >1500 ng/mL. Moreover, a 1500 ng/mL cutoff is predictive of VS (with a positive predictive value >90%), even more so than 300 ng/mL.

These findings imply that (1) the existing POC test, using a cutoff of 1500 ng/mL, could be leveraged to support adherence monitoring among patients on TAF, and (2) a single POC assay could be used for patients on all TFV-based ART. These results could likely also be extrapolated to TAF for prevention. Although messaging to individual patients would need to be clear that POC testing best captures more recent TAF dosing (within the last 24–48 hours, rather than out to 4–5 days as with TDF, based on modeling studies [4, 7]), our results suggest that patients on TAF who flag below 1500 ng/mL on POC testing may still benefit from enhanced adherence counseling, given strong associations with viremia.

Because self-reported ART adherence was high (with objective adherence metrics shown to be more reliable than self-report [9]), the availability of a POC tool to objectively measure adherence for patients on TFV-based regimens is useful. In settings in which frequent RNA monitoring is not feasible because of cost or access, POC testing could help clarify when virologic failures are more likely attributable to nonadherence (prompting interventions) or to resistance, requiring genotyping and potential ART change [9–11]. Even in settings in which frequent RNA checks are feasible, results are often not available in real time. Among underserved populations with HIV, including those experiencing homelessness or a digital divide, the ability to intervene more immediately to support adherence could prove valuable. Even among suppressed patients, drug level testing could help identify adherence challenges, prompting real-time adherence interventions aimed at improving the likelihood of continued VS, since suppressed patients with suboptimal adherence remain at risk of deleterious outcomes associated with inflammation [2].

Limitations of our study included low recruitment of women and that the timing of HIV RNA monitoring could not be standardized in this real-world sample. We also do not know with certainty whether a urine TFV cutoff of 1500 ng/mL would accurately predict protection against future HIV seroconversion for patients on TAF for preexposure prophylaxis, as shown for TDF [12]. Future research, including randomized trials, will be needed to assess the performance of the POC assay using standardized timeframes for RNA measurement.

Our study suggests that a single POC urine TFV assay with a 1500 ng/mL cutoff can be used to assess adherence predictive of VS for patients on TAF, like TDF. This assay can provide real-time data regarding the risk of HIV viremia, helping (1) inform counseling, (2) drive adherence interventions, and (3) improve the chances of achieving/maintaining VS.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. K. A. J. authored the initial draft of this manuscript and was responsible for all future revisions. K. A. J. and M. S. performed data analysis. H. O. and A. C. analyzed urine samples by liquid chromatography tandem mass spectrometry. M. A. and J. W. coordinated implementation of study procedures and patient recruitment. D. V. G. provided technical assistance on statistical methods. E. I. and M. D. H. provided subject matter expertise engaging patients vulnerable to adherence challenges. M. G. and M. S. assisted in project design and provided overall study oversight. All authors contributed substantively to the final version of the manuscript.

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Potential conflicts of interest. D. G. has consulting fees and accepted funds from Gilead Sciences. E. I. has received an honorarium for a speaking

engagement related to the POP-UP program at UCSF's Ward 86 HIV primary care clinic. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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