HHS Public Access

Author manuscript

AIDS. Author manuscript; available in PMC 2024 June 01.

Published in final edited form as:

AIDS. 2023 June 01; 37(7): 1109–1113. doi:10.1097/QAD.0000000000003520.

Point-of-care urine tenofovir testing to predict HIV drug resistance among individuals with virologic failure

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Abstract

Objective: We sought to evaluate the utility of a point-of-care (POC) urine tenofovir (TFV) assay, developed to objectively assess adherence, to predict HIV drug resistance (HIVDR) in people failing first-line antiretroviral therapy (ART).

Design: We retrospectively analyzed TFV levels as a biomarker of adherence in urine specimens collected during a clinical trial that enrolled adults with virologic failure on first-line ART in Uganda and South Africa.

Methods: Urine specimens were analyzed from participants on TFV-containing regimens who had a viral load >1,000 copies/mL and paired genotypic resistance test (GRT) results. We assessed recent ART TFV adherence with a qualitative POC lateral flow urine assay with a cut-off value

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of 1,500 ng/mL. We then calculated performance characteristics of the POC urine TFV assay to predict HIVDR, defined as intermediate or high-level resistance to any component of the current ART regimen.

Results: Urine specimens with paired plasma GRT results were available from 283 participants. The most common ART regimen during study conduct was emtricitabine, tenofovir disoproxil fumarate, and efavirenz. The overall prevalence of HIVDR was 86% (n=243/283). Of those with TFV detected on the POC assay, 91% (n=204/224) had HIVDR, versus only 66% (n=39/59) among those with no TFV detected (p-value<0.001). Positive and negative predictive values of the assay to predict HIVDR were 91% and 34%, respectively.

Conclusions: In populations with a high prevalence of HIVDR, the POC urine TFV assay can provide a low-cost, rapid method to guide requirements for confirmatory resistance testing and inform the need for regimen change.

Keywords

HIV; medication adherence; antiviral drug resistance; point-of-care test; urine; tenofovir

INTRODUCTION

Due to low availability of genotypic resistance testing (GRT) [1] in low- and middle-income countries (LMIC), treatment decisions after virologic failure are typically based on empirical guidelines [2]. This strategy has potential for unnecessary antiretroviral therapy (ART) regimen changes or inadvertent continuation of failing regimens.

Therapeutic drug monitoring is a potential tool for management of virologic failure by objectively evaluating ART adherence [3,4]. Although historically costly due to reliance on liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure drug levels [4], point-of-care (POC) urine tenofovir (TFV) assays have recently been developed to detect TFV using an antibody-based lateral flow assay. These assays, which function similarly to urine pregnancy tests, provide a low-cost, real-time measure of adherence [5–8]. POC urine TFV assays have been validated in laboratory settings and in populations on pre-exposure prophylaxis (PrEP) [6,9]. However, their utility as a clinical tool to assess the cause of virologic failure has not yet been fully evaluated.

METHODS

Study Design and Study Population

We conducted a diagnostic validity assessment utilizing specimens from the REVAMP clinical trial [10,11]. REVAMP enrolled adults ≥18 years at public-sector clinics in Uganda and South Africa who experienced virologic failure, defined as HIV-1 RNA viral load (VL) >1,000 copies/mL while on first-line ART from 2016 to 2019. Blood specimens were stored from each study visit. Urine collection and storage began one year after the study launch as part of a protocol amendment.

In this analysis, we included participants on TFV-containing first-line ART with stored urine and GRT results available from the same study visit. We selected the first applicable

specimen from each participant that met these criteria. REVAMP was approved by institutional review boards at Mbarara University of Science and Technology, University of KwaZulu-Natal, and Mass General Brigham, as well as the Uganda National Council of Science and Technology and the South African Department of Health. All participants gave written informed consent.

Laboratory Methods

GRT was performed using Sanger sequencing for plasma specimens with a VL >1,000 copies/mL [11]. We defined HIVDR as intermediate or high-level resistance to any drug in the current ART regimen, as determined by the Stanford algorithm [12].

Cryopreserved urine specimens were tested at the Africa Health Research Institute Pharmacology Core Laboratory using a POC lateral flow assay, which classifies TFV as present versus (vs) absent with a cut-off of 1,500 ng/mL [6]. The urine TFV assay provides a measure of short-term adherence over the prior four to seven days and has been previously validated against methods using LC-MS/MS [5,6].

Statistical Analysis

We compared the percentage of participants with HIVDR among those with vs without TFV in urine overall, as well as stratified by VL (> vs < 10,000 copies/mL), age (> vs < 40 years), and duration of ART (> vs < 4 years). We also conducted an exploratory analysis to evaluate factors associated with absence of HIVDR among those with TFV present in urine. We calculated test performance characteristics of the POC urine TFV assay to predict HIVDR by estimating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with exact 95% confidence intervals (CI). We also estimated PPVs and NPVs across a range of HIVDR prevalence estimates. Finally, we compared performance of the urine TFV assay with self-reported adherence within the past month (perfect vs imperfect) to predict HIVDR. Data analysis was performed using Stata v14 (College Station, Texas).

RESULTS

Study Population and Characteristics

Of 840 participants in REVAMP, we analyzed urine specimens with paired GRT results available from 283 participants on TFV-containing regimens (Supplemental Figure 1). We excluded participants from this analysis who were on non-TFV regimens, did not have urine collected, or did not have GRT results available. Excluded participants had a higher median age and longer median duration of ART, compared to those who were included.

Median age at study enrollment was 35 years (interquartile range [IQR]: 30 – 43 years), and 53% (151/283) were female. Ninety-five percent (269/283) were on lamivudine (or emtricitabine, XTC), tenofovir disoproxil fumarate (TDF), and efavirenz; while 5% were on XTC, TDF, and nevirapine. Median duration of ART was 4.6 years (IQR: 3.3 – 6.7 years). Most specimens analyzed (277/283, 98%) were from the study enrollment visit. Prevalence of HIVDR was 86% (243/283). Among those with HIVDR, nearly all (242/243, 99.6%)

had resistance to NNRTIs, and 83% (202/243) had resistance to both nucleoside reverse transcriptase inhibitors and NNRTIs. Only one participant had M184V alone.

HIVDR in Participants with versus without Detectable TFV in Urine

Among participants with TFV in urine (all of whom had VL >1,000 copies/mL, based on study inclusion criteria), 91% (204/224) had HIVDR vs 66% (39/59) of those without TFV in urine ($p<0.001$; Figure 1). This relationship was generally similar when stratified by VL, age, and duration of ART, although the urine TFV assay had better discriminatory value with those under 40 (PPV 94% [95% CI: 89% – 97%]; NPV 38% [95% CI: 23% – 54%]) versus those older than 40 years of age (PPV 86% [95% CI: 75% – 93%]; NPV 26% [95% CI: 9% – 51%]) (Figure 1).

Twenty participants had detectable TFV and no HIVDR, all of whom contributed specimens from the study enrollment visit. Older age was associated with absence of HIVDR among those with detectable TFV (Wilcoxon rank sum, $p=0.0142$). However, no other factors differentiated that group in exploratory analyses, including sex, duration of ART, ART regimen, and VL.

Test Performance Characteristics

Overall, the POC urine TFV assay had a sensitivity of 84% (95% CI: 79% - 88%) and specificity of 50% (95% CI: 34% - 66%) to predict HIVDR in participants with viremia (Table 1A). Based on the study prevalence of HIVDR of 86%, PPV was 91% (95% CI: 87% - 95%), and NPV was 34% (95% CI: 22% - 47%) (Table 1A). At 90% prevalence of HIVDR, the PPV is expected to be 94% (95% CI: 92% - 95%) and the NPV 26% (95% CI: 19% – 35%, Table 1B). As prevalence of HIVDR decreases, PPV of the assay is also expected to decrease, but NPV would increase, such that at 10% prevalence of HIVDR, the NPV is expected to reach 97% (95% CI: 95% - 98%, Table 1B).

Self-reported perfect vs imperfect adherence had a sensitivity of 18% (95% CI: 13% - 23%) and specificity of 83% (95% CI: 67% - 93%) to predict HIVDR in participants with viremia. PPV was 86% (95% CI: 73% - 94%), and NPV was 14% (95% CI: 10% - 19%).

DISCUSSION

The real-time urine TFV assay exhibited a PPV of 91% to predict HIVDR in a population failing NNRTI-based ART with a high (86%) prevalence of resistance. Conversely, NPV was much lower in this population, and a negative urine TFV assay alone thereby would not reliably exclude HIVDR in a setting with a high prevalence of resistance. By comparison, assuming similar performance characteristics, the assay is predicted to have high NPV in settings with low HIVDR prevalence, as might be expected with integrase strand transfer inhibitor (INSTI)-based ART [13,14], the now-preferred first-line regimen throughout sub-Saharan Africa [2]. Moreover, the urine TFV assay outperformed self-reported adherence to predict HIVDR, with respect to sensitivity, PPV, and NPV, thus suggesting value over historically unreliable self-reported adherence metrics [15,16].

Advantages of urine adherence assays include low costs and point-of-care availability. Few studies have evaluated the utility of urine ART levels to determine the etiology of virologic failure in resource-limited settings. A recent pilot study in South Africa (n = 14) similarly reported high sensitivity (100%) for this POC urine TFV assay to predict HIVDR [17]. In addition, a study evaluating detectable plasma protease inhibitor levels, measured by LC-MS/MS, revealed high sensitivity and high NPV for detectable ART levels to predict resistance in a sample with a lower prevalence of HIVDR (27%) [18]. Another study found that intermediate levels of tenofovir-diphosphate (TFV-DP) in dried blood spots, a longer-term measure of adherence, were associated with HIVDR in participants with viremia, as compared to lower levels of TFV-DP for those without HIVDR [19]. These findings are consistent with ours, which suggest that an objective metric of adherence has high PPV for HIVDR in settings with high HIVDR prevalence. Notably, in our study with a much larger sample, the presence of TFV had a high but imperfect probability of predicting HIVDR (91%), suggesting that some individuals taking therapy retain activity to their regimen. This was seen more commonly in older participants, perhaps reflecting more consistent longer-term adherence for this group. Conversely, we also found that the TFV assay will be expected to have a high NPV for HIVDR in scenarios where HIVDR prevalence to first-line regimens is expected to be low, such as with the current dolutegravir-based regimens recommended by the World Health Organization, which was also seen in a recent study evaluating use of this assay in a population on high genetic barrier regimens [20].

Our findings should be interpreted considering study limitations. This study was conducted using retrospective analysis of specimens in a centralized laboratory and only included participants on NNRTI-based regimens. In addition, the POC urine TFV assay provides qualitative information on recent adherence but does not provide information on longer-term adherence beyond seven days [6] or on gradations of adherence. Assays for short-term adherence may also be subject to “white coat adherence”, in which medications may be taken in anticipation of a clinic visit [21]. We do not suspect “white coat adherence” to be a source of bias in this study, because 98% of specimens analyzed were from the study enrollment visit before participants were aware of urine drug monitoring. We consider this ability to exclude white coat adherence and the large sample size the two greatest strengths of this study. Future work should explore use of the urine TFV assay in individuals taking dolutegravir-based regimens as a trigger for adherence interventions to increase virologic suppression rates.

In summary, with a low expected cost [6], this POC urine TFV assay holds promise for a wide range of applications for ART programs in LMICs. In settings with a high prevalence of HIVDR (such as populations failing NNRTI-based regimens), the POC urine TFV assay could provide a low-cost method to focus HIVDR testing or to assist in informing regimen changes. Conversely, the assay could assist with excluding HIVDR and triggering immediate adherence interventions in settings with low HIVDR prevalence, as may be expected in populations taking dolutegravir-based regimens, where adherence challenges (rather than dolutegravir resistance) are currently expected to underlie most cases of virologic failure [20]. Future field-based studies are needed to evaluate prospective implementation of the POC urine TFV assay among people with virologic failure in sub-Saharan Africa. Such studies can provide guidance on the need for additional GRT testing, the utility of changing

ART regimens, and triggering real-time adherence interventions based on a low-cost metric, depending on the regimen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We would like to acknowledge the REVAMP study participants and staff for their dedication to this work. S.M.M., K.G., J.A., M.G., M.A.S., M.Y.M., W.M., P.M., H.S., B.H.G., V.C.M., and M.J.S contributed to the study design. S.M.M., W.M., M.Y.M., G.M., H.S., S.P., and M.J.S oversaw data and specimen collection. M.P. and G.M. processed the laboratory specimens for the study and ensured selection of appropriate specimens for analysis. K.G. conducted the urine tenofovir assays for the study. M.G. and M.A.S contributed to development of the urine tenofovir assay, provided consultation on interpretation of the urine tenofovir assays, and provided expertise on objective adherence measures. S.M.M., K.G., J.A., S.P., and G.C. curated the study data and conducted data quality checks. S.M.M. conducted the data analysis and drafted the manuscript. B.H.G. and M.J.S. provided consultation and oversight of the statistical analysis. All authors contributed to critical review of the manuscript.

FUNDING:

This work was supported by the National Institutes of Health [R21AI145537 to S.M.M, R01AI124718 to M.J.S and W.M, R01AI143340 to M.G., and K23MH122286 to M.S.], the Gilead Sciences Research Scholars Program in HIV [research grant to S.M.M], the Emory University Center for AIDS Research [P30AI050409 to V.C.M] and the Harvard University Center for AIDS Research P30AI060354 to S.M.M.]. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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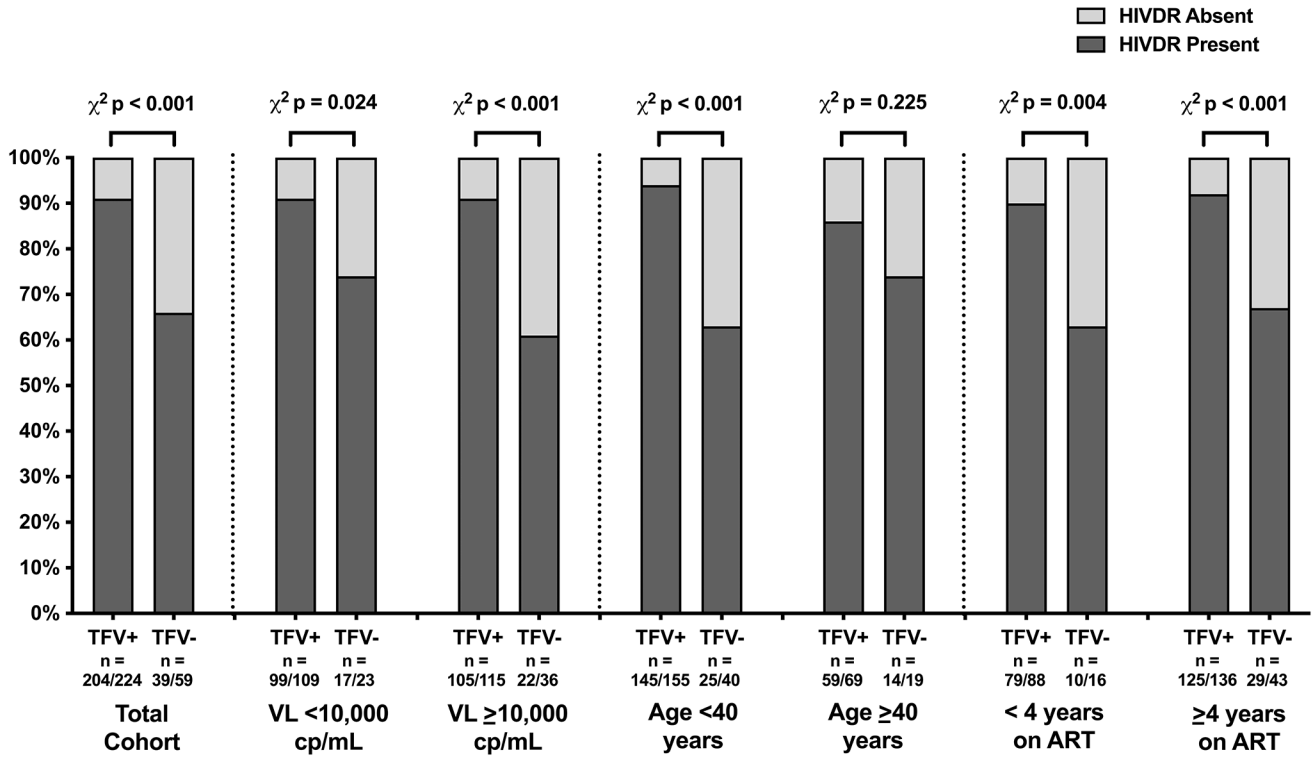

FIGURE 1.

Proportion with HIV Drug Resistance in Participants with and without Detectable Urine Tenofovir, Stratified by HIV-1 RNA Viral Load, Age, and Duration of Antiretroviral Therapy. We calculated the proportion with HIV drug resistance among participants with and without detectable tenofovir in urine using the point of care assay, stratified by HIV-1 RNA viral load, age, and duration of ART. Using the Stanford algorithm [12], HIV drug resistance was defined as intermediate to high-level resistance to any component of the current antiretroviral therapy regimen. TFV=tenofovir, VL=viral load, ART=antiretroviral therapy, HIVDR=HIV drug resistance

TABLE 1.

Test Characteristics of a Point-of-Care Urine Tenofovir Assay to Predict HIV Drug Resistance

A. Test Characteristics		**B. HIVDR Prevalence Estimates**	**PPV**	**NPV**
	% (95% CI)		**% (95% CI)**	**% (95% CI)**
Sensitivity	84 (79, 88)	90%	94 (92, 95)	26 (19, 35)
Specificity	50 (34, 66)	75%	83 (79, 87)	51 (41, 61)
PPV	91 (87, 95)	50%	63 (55, 70)	76 (67, 83)
NPV	34 (22, 47)	25%	36 (29, 43)	90 (86, 93)
Observed HIVDR Prevalence	*86 (81, 90)*	10%	16 (12, 20)	97 (95, 98)