Title

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Journal

JAIDS Journal of Acquired Immune Deficiency Syndromes, 88(1)

ISSN

1525-4135

Authors

Johnson, Kelly A
Okochi, Hideaki
Glidden, David V
et al.

Publication Date

2021-09-01

DOI

10.1097/qai.0000000000002727

Peer reviewed

Published in final edited form as:

J Acquir Immune Defic Syndr. 2021 September 01; 88(1): 57–60. doi:10.1097/QAI.0000000000002727.

Brief Report: No Difference in Urine Tenofovir Levels in Patients Living with HIV on Unboosted vs Dose-Adjusted Boosted Tenofovir Alafenamide

Kelly A. Johnson[1], **Hideaki Okochi**[1], **David V. Glidden**[2], **Monica Gandhi**[1], **Matthew Spinelli**[1]

[1]Department of Medicine, University of California San Francisco, San Francisco, CA

[2]Department of Epidemiology & Biostatistics, University of California San Francisco, San Francisco, CA

Abstract

Background: Tenofovir alafenamide (TAF) is increasingly used in HIV treatment, with or without agents that require pharmacologic boosters like ritonavir/cobicistat. Boosters increase TAF levels, so the TAF dose is lowered in single pill combinations. We hypothesized that individuals on dose adjusted boosted TAF would have similar urine tenofovir (TFV) concentrations to those on unboosted TAF.

Setting/Methods: We collected urine samples from patients with HIV on TAF with evidence of virologic suppression and high self-reported adherence at two San Francisco clinics from June 2019-January 2020. We measured urine TFV levels by liquid chromatography/tandem mass spectrometry and used linear regression to compare natural log-transformed urine TFV levels for patients on boosted versus unboosted TAF.

Results: Our analysis included 30 patients on unboosted TAF (25mg daily) and 15 on boosted TAF (12 on 10mg daily TAF, 3 on 25mg). Patients on unboosted vs. boosted TAF had similar baseline age, weight, gender, and creatinine. In unadjusted univariate linear regression, there were no significant differences in urine TFV levels based on presence/absence of boosting following TAF dose-reduction to 10mg [geometric mean ratio 1.07; 95% CI: 0.53–2.16]. This finding was unchanged in adjusted analysis.

Conclusions: No significant differences in urine TFV levels were seen for patients on unboosted vs. boosted dose reduced TAF. These results have important implications for our forthcoming point-of-care urine immunoassay for TAF, implying that separate adherence cut-offs will not be necessary for patients on boosters and dose-reduced TAF. A single POC TAF immunoassay will thus support monitoring on most TAF-based antiretroviral therapy.

Corresponding author: Kelly A. Johnson, MD, MPH; 513 Parnassus Ave., Rm S380, San Francisco CA, 94143; 757.725.3254; kjohnson@ucsf.edu. **Alternate corresponding author:** Matthew Spinelli, MD, MAS; 995 Potrero Ave, Building 80, Ward 84, San Francisco, CA 94110; 201.247.9124; matthew.spinelli@ucsf.edu.

Conflicts of Interest:
Dr. D. Glidden has accepted funds from Gilead Sciences.

Keywords

Adherence; Point-of-care Monitoring; Anti-retroviral therapy; Tenofovir Alafenamide; Pharmacokinetics

Background:

For people living with HIV, virologic suppression and disease control rely on adherence, currently to daily antiretroviral therapy (ART),[1] to achieve optimal efficacy. Both pre-exposure prophylaxis (PrEP) and HIV treatment as prevention – the concept that undetectable HIV viral load equals untransmittable virus[2-5] – require adequate adherence and are key strategies for ending the HIV epidemic.[6]

ART adherence could be supported by point-of-care (POC) testing of drug levels to provide real-time feedback – particularly in settings where frequent HIV viral load monitoring may not be feasible and/or where adherence data could inform clinical counseling messages or prompt adherence interventions when necessary. Studies have shown that such objective pharmacologic metrics of adherence – with drug levels measured in biomatrices such as dried blood spots (DBS), plasma, hair or urine – more accurately predict antiretroviral efficacy than self-report, which is subject to limitations such as social desirability and recall biases.[7-12]

Traditional pharmacologic adherence metrics typically rely on liquid chromatography/tandem mass spectrometry (LC-MS/MS), which requires specialized equipment, personnel, and is expensive.[1] However, we previously developed and validated a low-cost, easy-to-perform, rapid turnaround antibody-based assay to measure urine levels of the metabolite tenofovir (TFV) as a short-term metric of adherence in patients taking the antiretroviral tenofovir disoproxil fumarate (TDF).[13-15] When tested against the analytic gold standard LC-MS/MS, this assay has been found to be highly sensitive and specific (97-99%)[1] with a high degree of correlation between the two techniques (0.92, $p<0.001$).[14] The enzyme-linked immunoassay has now been converted to a lateral flow assay (LFA), similar to a urine pregnancy test, allowing POC testing of the presence or absence of recent TDF dosing (providing a qualitative yes/no response as to whether TDF has been taken within the last five days), thereby supporting real-time adherence feedback in routine clinical settings and at the patient bedside.[1,13]

An alternate tenofovir prodrug, tenofovir alafenamide (TAF), is now commonly used in HIV treatment regimens and has been recently approved as a component of PrEP in certain populations.[16] TDF is metabolized to TFV in the gut and plasma, whereas TAF is metabolized to TFV in the peripheral blood mononuclear cells; both are concentrated/excreted in the urine.[17,18] Because TAF is primarily metabolized intracellularly, TFV levels in the plasma are approximately 90% lower with TAF than with TDF.[19] Urine levels are also expected to be lower[18,20]– necessitating separate adherence cut-offs with our forthcoming urine immunoassay for use in patients on TAF.

In HIV treatment regimens, TAF is typically dose reduced from 25 mg to 10 mg when given in single pill combinations with cobicistat, a pharmacologic booster which increases the levels of TAF and its metabolites. Ritonavir is also a booster but its use may become less common, as it has not been coformulated in single pill regimens.[21] In this study, we hypothesized that individuals on dose-adjusted/reduced TAF with boosting would have similar urine TFV concentrations compared to those on unboosted TAF, a finding with important implications for the implementation of point-of-care urine immunoassays for use in patients living with HIV on TAF-based ART.

Settings/Methods:

We collected urine samples from patients living with HIV receiving primary care services at two San Francisco clinics from June 2019-January 2020. Interested participants were recruited via flyers and met with a study coordinator to: (1) determine eligibility for this study, (2) if eligible, answer a questionnaire regarding self-reported ART adherence (including number of ART doses missed, percentage of the time antiretrovirals were taken in the last 7 days, and number of days since last ART dosing), and (3) provide a urine sample. Patients were considered eligible if they were: at least 18 years old, on TAF-based ART by self-report and medical record, able to give written informed consent in English, and able to provide at least one ounce of urine. We measured urine TFV levels by LC-MS/MS in the Hair Analytical Laboratory at the University of California San Francisco (UCSF) using methods validated in house for urine and akin to those previously published for hair.[14,15,22]

We also reviewed each study participant's electronic medical record to confirm their ART regimen – including TAF dose (25 mg vs 10 mg) and presence/absence of pharmacologic booster – and to collect additional clinical variables (all at or around the time of urine sample collection) such as age, gender, weight, creatinine (most recent within six months before or after urine collection), and HIV viral load (most recently documented viral load around urine sample collection). We restricted this analysis to patients with viral suppression (most recent HIV RNA <200 copies/ml) who also self-reported high adherence to ART, including having taken their antiretrovirals within the last 24 hours, on the study questionnaire.

In both unadjusted and adjusted models, we used linear regression to compare natural log-transformed urine TFV levels for patients on TAF plus a booster to those on unboosted TAF. Baseline clinical characteristics were compared for patients on boosted versus unboosted TAF using χ^2 or Fisher exact testing for categorical variables and Wilcoxon rank-sum test for continuous variables. All statistical analyses were performed using STATA version 16.0. This study was reviewed and approved by the Institutional Review Board of UCSF.

Results:

After excluding patients (n=3) whose most recent HIV viral load around the time of urine sample collection was >200 copies/ml, our analysis included one sample each from 30 patients on unboosted TAF (25 mg daily) and 15 on boosted TAF (12 on 10 mg daily TAF,

3 on 25 mg). Cobicistat was the most frequently used booster, with only one patient on a ritonavir-containing regimen with TAF at 25 mg.

Patients on unboosted vs. boosted TAF (Table 1) were similar in baseline median age (56 vs 54 years p=0.69), weight (84 vs 80 kg; p=0.47), creatinine (1.1 vs 1.0 mg/dL; p=0.21), and gender (83% and 80% cis male; p=0.85). There was one transgender female (assigned male sex at birth) and one transgender male (assigned female sex at birth), both of whom were in the unboosted TAF group and neither of whom was on hormone replacement therapy. The median (IQR) urine TFV levels by LC-MS/MS were 4.14 (2.03, 6.56) and 4.27 μg/mL (2.73, 5.51) in the unboosted and boosted TAF groups, respectively.

In unadjusted univariate linear regression (Supplemental Table 1), there were no statistically significant differences in urine TFV levels based on presence/absence of a booster following TAF dose-reduction to 10 mg [geometric mean ratio (GMR) 1.07; 95% CI: 0.53-2.16]. This finding was unchanged in adjusted analysis accounting for sex assigned at birth (with male sex as the reference category), age, creatinine, and weight [GMR 1.10; 95% CI: 0.53-2.28 (Table 2)].

Due to the smaller sample size (with only three patients falling into this category), we cannot exclude a clinically significant increase in urine TFV levels in the boosted TAF 25 mg group (GMR 1.46; 95% CI: 0.41- 5.12).

Discussion/Conclusions:

We found no clinically significant differences in urine tenofovir levels for patients on unboosted (25 mg) vs cobicistat-boosted dose-reduced TAF (10mg). This finding mirrors what is seen in the plasma, where boosted 10 mg TAF and unboosted 25 mg TAF produce similar plasma TFV exposures, [23,24] leading to Food and Drug Administration approval for a TAF dose of 10 mg when co-formulated with cobicistat in the single pill regimens of elvitegravir / cobicistat / emtricitabine / tenofovir alafenamide[25] and darunavir / cobicistat / emtricitabine / tenofovir alafenamide.[26]

Our results have important implications for our forthcoming POC immunoassay for TAF, implying that separate adherence cut-offs will not be necessary for patients on boosted TAF with dose reduction compared with patients on unboosted 25 mg TAF. A single POC TAF urine immunoassay will thus support adherence monitoring for patients on TAF-based PrEP and most TAF-based ART, whether at the standard 25 mg unboosted dose or the cobicistat-boosted 10 mg dose.

Such real-time adherence monitoring and feedback via drug level measurement will be clinically useful for patients living with HIV, most of whom are on tenofovir based regimens, particularly in settings where routine or frequent viral load monitoring and/or resistance testing may not be feasible. In a recent South Africa study of HIV positive persons on second line lopinavir (LPV)-based ART, for instance, most cases of virologic failure were attributable to non-adherence – demonstrated by undetectable LPV levels in plasma and DBS – as opposed to drug resistance mutations. No patients with undetectable LPV plasma levels were found to have LPV resistance by genotypic analysis.[27] This

finding suggests that strategies to objectively measure adherence could prove invaluable in determining when virologic failure in patients with HIV is more likely due: (A) to non-adherence, prompting adherence support or interventions, or (B) to drug resistance mutations, prompting resistance testing and/or switch to an alternative treatment regimen.[27]

Even in settings where viral load monitoring and resistance testing are more readily available and/or affordable, regular POC drug level testing, with subsequent adherence support where needed, could lead to higher rates of virologic suppression among patients with HIV facing adherence challenges.[1] Our present research implies that two POC urine immunoassays – one for TAF and another for TDF – could support such adherence monitoring in real-time for patients on tenofovir-based antiretrovirals.

Limitations of our study include the lack of directly-observed-therapy to confirm recent TFV exposure at the time of urine sample collection. All patients in this analysis, however, reported excellent ART adherence and had documented HIV viral loads <200 copies/ml by chart review. We were unable to measure pharmacokinetic parameters, as timed washout studies were not feasible in this population taking daily ART for HIV treatment. Lastly, due to the small sample size among patients taking 25 mg TAF plus a booster (with only three patients in this group), we are unable to exclude a clinically significant increase in urine TFV levels in these participants.

In conclusion, our future POC urine immunoassays for TDF and now TAF have the potential to serve as important clinical tools, providing real-time adherence feedback to providers and patients in routine clinical settings, thereby informing patient/provider communication, counseling messages, and adherence support interventions. In the case of the TAF POC assay, efforts are currently underway to establish the TFV cut-offs that reliably predict recent TAF exposure. This current study shows that the same TAF assay can be used for those on dose-adjusted boosted and unboosted TAF. Future studies will explore the correlation of urine TFV levels via our immunoassay with viremic control in patients on TAF-based ART and real time adherence monitoring for those on TAF-based antiretrovirals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding:

This work was supported, in part, by the National Institute of Mental Health (K23MH122286, Dr. M. Spinelli) and the National Institute of Allergy and Infectious Diseases (R01AI152119, Dr. M. Gandhi). The manuscript was also made possible in part through support from the AIDS Research Institute at UCSF (Dr. K. Johnson).

References:

1. Spinelli MA, Haberer JE, Chai PR, Castillo-Mancilla J, Anderson PL, Gandhi M. Approaches to objectively measure antiretroviral medication adherence and drive adherence interventions. Curr HIV/AIDS Rep. 2020;17(4):301–314. [PubMed: 32424549]
2. Cohen MS, Chen YQ, McCauley M, et al.Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011;365(6):493–505. [PubMed: 21767103]

3. Cohen MS, Chen YQ, McCauley M, et al.Antiretroviral Therapy for the Prevention of HIV-1 Transmission. N Engl J Med. 2016;375(9):830–839. [PubMed: 27424812]
4. Rodger AJ, Cambiano V, Bruun T, et al.Sexual Activity Without Condoms and Risk of HIV Transmission in Serodifferent Couples When the HIV-Positive Partner Is Using Suppressive Antiretroviral Therapy. JAMA. 2016;316(2):171–181. [PubMed: 27404185]
5. Rodger AJ, Cambiano V, Bruun T, et al.Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): final results of a multicentre, prospective, observational study. Lancet. 2019;393(10189):2428–2438. [PubMed: 31056293]
6. Fauci AS, Redfield RR, Sigounas G, Weahkee MD, Giroir BP. Ending the HIV epidemic: a plan for the United States. Jama. 2019;321(9):844–845. [PubMed: 30730529]
7. Anderson PL, Glidden DV, Liu A, et al.Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. Sci Transl Med. 2012;4(151):151ra125.
8. Marrazzo JM, Ramjee G, Richardson BA, et al.Tenofovir-based preexposure prophylaxis for HIV infection among African women. N Engl J Med. 2015;372(6):509–518. [PubMed: 25651245]
9. Grant RM, Lama JR, Anderson PL, et al.Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Engl J Med. 2010;363(27):2587–2599. [PubMed: 21091279]
10. Liu AY, Yang Q, Huang Y, et al.Strong relationship between oral dose and tenofovir hair levels in a randomized trial: hair as a potential adherence measure for pre-exposure prophylaxis (PrEP). PLoS One. 2014;9(1):e83736. [PubMed: 24421901]
11. Anderson PL, Liu AY, Castillo-Mancilla JR, et al.Intracellular tenofovir-diphosphate and emtricitabine-triphosphate in dried blood spots following directly observed therapy. Antimicrob Agents Chemother. 2018;62(1).
12. Koss CA, Bacchetti P, Hillier SL, et al.Differences in cumulative exposure and adherence to tenofovir in the VOICE, iPrEx OLE, and PrEP demo studies as determined via hair concentrations. AIDS Res Hum Retroviruses. 2017;33(8):778–783. [PubMed: 28253024]
13. Gandhi M, Wang G, King R, et al.Development and validation of the first point-of-care assay to objectively monitor adherence to HIV treatment and prevention in real-time in routine settings. Aids. 2020;34(2):255–260. [PubMed: 31634188]
14. Gandhi M, Bacchetti P, Spinelli MA, et al.Brief report: validation of a urine tenofovir immunoassay for adherence monitoring to PrEP and ART and establishing the cutoff for a point-of-care test. J Acquir Immune Defic Syndr. 2019;81(1):72–77. [PubMed: 30664078]
15. Gandhi M, Bacchetti P, Rodrigues WC, et al.Development and validation of an immunoassay for tenofovir in urine as a real-time metric of antiretroviral adherence. EClinicalMedicine. 2018;2-3:22–28. [PubMed: 30906930]
16. Hare CB, Coll J, Ruane R, et al.The Phase 3 DISCOVER study: daily F/TAF or F/TDF for HIV preexposure prophylaxis. Presented at: Conference on Retroviruses and Opportunistic Infections 2019; Seattle, WA.
17. Wassner C, Bradley N, Lee Y. A review and clinical understanding of tenofovir: tenofovir disoproxil fumarate versus tenofovir alafenamide. J Int Assoc Provid AIDS Care. 2020;19:2325958220919231.
18. Lalley-Chareczko L, Hiserodt E, Moorthy G, Zuppa A, Mounzer K, Koenig H. Urine assay to measure tenofovir concentrations in patients taking tenofovir alafenamide. Front Pharmacol. 2020;11:286. [PubMed: 32265700]
19. Podany AT, Bares SH, Havens J, et al.Plasma and intracellular pharmacokinetics of tenofovir in patients switched from tenofovir disoproxil fumarate to tenofovir alafenamide. Aids. 2018;32(6):761–765. [PubMed: 29334548]
20. Haaland RE, Martin A, Livermont T, et al.Brief report: urine emtricitabine and tenofovir concentrations provide markers of recent antiretroviral drug exposure among HIV-negative men who have sex with men. J Acquir Immune Defic Syndr. 2019;82(3):252–256. [PubMed: 31335590]
21. Tseng A, Hughes CA, Wu J, Seet J, Phillips EJ. Cobicistat versus ritonavir: similar pharmacokinetic enhancers but some important differences. Ann Pharmacother. 2017;51(11):1008–1022. [PubMed: 28627229]

22. Okochi H, Louie A, Phung N, et al.Tenofovir and emtricitabine concentrations in hair are comparable between individuals on tenofovir disoproxil fumarate versus tenofovir alafenamide-based ART. Drug Test Anal. 2021.

23. Zack J, Chuck S, Chu H, et al.Bioequivalence of the rilpivirine/emtricitabine/tenofovir alafenamide single-tablet regimen. Journal of Bioequivalence & Bioavailability. 2016;8:1–6.

24. Ogbuagu O. Rilpivirine, emtrictiabine, and tenofovir alafenamide: single-table combination for the treatment of HIV-1 in selected patients. Expert Rev Anti Infect Ther. 2016;14(12):1113–1126. [PubMed: 27797606]

25. Food and Drug Administration (FDA). Full prescribing information: genvoya (elvitegravir, cobicistat, emtricitabine, and tenofovir alafenamide). Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/207561s000lbl.pdf.Accessed December 8, 2020.

26. Food and Drug Administration (FDA). Full prescribing information: symtuza (darunavir, cobicistat, emtricitabine, and tenofovir alafenamide). Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/210455s009lbl.pdf.Accessed December 8, 2020.

27. Hermans LE, Steegen K, Ter Heine R, et al. Drug level testing as a strategy to determine eligibility for drug resistance testing after failure of ART: a retrospective analysis of South African adult patients on second-line ART. J Int AIDS Soc. 2020;23(6):e25501. [PubMed: 32515898]

Table 1:

Baseline Characteristics and Urine Tenofovir (TFV) Levels in People Living with HIV with Virologic Suppression on Tenofovir Alafenamide (TAF)-based Antiretroviral Therapy

	No Booster	**Booster**
Number of Patients	30	15
Age (years), median (IQR)	56 (45, 61)	54 (47, 60)
Gender		
Cis Female	3 (10%)	3 (20%)
Cis Male	25 (83%)	12 (80%)
Transgender Male	1 (3%)	0 (0%)
Transgender Female	1 (3%)	0 (0%)
Weight (kg), median (IQR)	83.9 (73.5, 96.7)	79.9 (73.9, 85.7)
Creatinine (mg/dL), median (IQR)	1.1 (0.9-1.3)	1.0 (0.9-1.2)
TAF dose		
10 mg	0 (0%)	12 (80%)
25mg	30 (100%)	3 (20%)
LC-MS/MS results (urine TFV level, μg/mL), median (IQR)	4.14 (2.03, 6.56)	4.27 (2.73, 5.51)

Abbreviation: LC-MS/MS = liquid chromatography/tandem mass spectrometry

Table 2:

Adjusted Linear Regression Analysis for Boosted vs. Unboosted Tenofovir Alafenamide (TAF) and Urine Tenofovir Levels by LC-MS/MS in People Living with HIV with Virologic Suppression on TAF-based Antiretroviral Therapy

Variable	Geometric mean ratio	Standard Error	95% Confidence Interval	P-value
TAF dose				
Unboosted TAF (25 mg)	reference	--	--	--
Boosted TAF (10 mg)	1.10	0.36	0.53- 2.28	0.80
Boosted TAF (25 mg)	1.46	0.62	0.41- 5.12	0.55
Creatinine (mg/dL)	1.32	0.61	0.38-4.56	0.65
Age per ten years	0.82	0.14	0.62- 1.08	0.16
Sex assigned at birth *	1.35	0.34	0.68- 2.70	0.38
Natural log-transformed weight in kilograms	0.57	0.73	0.13- 2.51	0.45

* Reference group: male sex assigned at birth

Abbreviation: LC-MS/MS = liquid chromatography/tandem mass spectrometry