Clinical Infectious Diseases

MAJOR ARTICLE

Tenofovir Diphosphate in Dried Blood Spots Is Strongly Associated With Viral Suppression in Individuals With Human Immunodeficiency Virus Infections

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Background. Although tenofovir diphosphate (TFV-DP) in dried blood spots (DBS) is a predictor of adherence and pre-exposure prophylaxis efficacy, its utility in human immunodeficiency virus (HIV) treatment remains unknown.

Methods. DBS for TFV-DP were collected up to 3 times over 48 weeks in persons living with HIV (PLWH) who were receiving TFV disoproxil fumarate (TDF)-based therapy. Log-transformed baseline TFV-DP was compared using *t*-tests or analyses of variance; generalized estimating equations were used to estimate the adjusted odds ratio (aOR) of viral suppression (<20 copies/mL) based on the TFV-DP concentration at the study visit.

Results. We analyzed 1199 DBS from 532 participants (76 female; 101 Black, 101 Hispanic). Among the virologically-suppressed participants at baseline (n = 347), TFV-DP was lower in Blacks (geometric mean 1453, 95% confidence interval [CI] 1291–1635) vs Whites (1793, 95% CI 1678–1916; $P = .002$) and Hispanics (1760, 95% CI 1563–1982; $P = .025$); in non-boosted (1610, 95% CI 1505–1723) vs. boosted (1888, 95% CI 1749–2037; $P = .002$) regimens; and in non-nucleoside reverse transcription inhibitor–based (1563, 95% CI 1432–1707) vs. boosted protease inhibitor–based (1890, 95% CI 1704–2095; $P = .006$) and multiclass-based (1927, 95% CI 1650–2252; $P = .022$) regimens. The aOR of virologic suppression, after adjusting for age, gender, race, body mass index, estimated glomerular filtration rate, $CD4^+$ T-cell count, antiretroviral drug class and duration of therapy, was 73.5 (95% CI 25.7–210.5; $P < .0001$) for a TFV-DP concentration ≥1850 fmol/punch compared to <350 fmol/punch.

Conclusions. TFV-DP in DBS is strongly associated with virologic suppression in PLWH on TDF-based therapy and is associated with certain participant characteristics. Further research is required to evaluate this drug adherence and exposure measure in clinical practice.

Clinical Trials Registration: NCT02012621.

Keywords. adherence; dried blood spots; tenofovir diphosphate; antiretroviral therapy; pharmacokinetics.

The main driver of antiretroviral therapy (ART) exposure and human immunodeficiency virus (HIV)-related outcomes is medication adherence [1–3]. Optimal adherence is critical to achieve and sustain viral suppression in people living with HIV (PLWH) [4, 5]. Despite this, we currently lack a gold standard measure of ART adherence and exposure that can be used in clinical practice [6, 7]. In particular, measures that distinguish between viremia due to suboptimal adherence vs inadequate viral response (eg, persistent viremia due to drug resistance or low cumulative drug exposure despite high adherence) are currently unavailable.

Tenofovir diphosphate (TFV-DP), arising from tenofovir disoproxil fumarate (TDF)- and tenofovir alafenamide (TAF)-based regimens [8, 9], is the phosphorylated anabolite of tenofovir (TFV) and exerts its pharmacodynamic effect in HIV-infected cells. This anabolite is also abundant in red blood cells [10, 11] and dried blood spots (DBS) [11–13], where it exhibits unique pharmacology, with a long half-life of 17 days and a 25-fold accumulation from the first dose to steady state [11]. These distinctive pharmacologic characteristics have been leveraged to develop an adherence gradient in HIV-negative persons [11, 14] that is predictive of HIV pre-exposure prophylaxis (PrEP) efficacy [15–17] and correlates with other adherence measures in HIV [18, 19]. However, this biomarker as an indicator of viral suppression and drug exposure in PLWH on ART has not been evaluated. This is a significant gap in knowledge given the >20 million PLWH receiving ART worldwide [20] (many of whom are on TFV-based regimens in the United States [>85%] [21] and around the world [22]) and the proportion in whom viral suppression has not been achieved [23].

Received 11 May 2018; editorial decision 26 July 2018; accepted 21 August 2018; published online August 23, 2018.

Presented in part: Conference on Retroviruses and Opportunistic Infections, Boston, MA, 5–8 March 2018. Abstract number 25.

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Clinical Infectious Diseases® 2019;68(8):1335–42

DOI: 10.1093/cid/ciy708

We therefore investigated the association of TFV-DP with viral suppression in a clinical cohort of PLWH being treated with TDF-based ART.

METHODS

Study Design and Participants

Study participants were recruited at the University of Colorado Hospital Infectious Diseases Group Practice in Aurora, Colorado. Inclusion criteria required that participants were 18 years or older, taking a TDF-based regimen (for any duration of time), and planned to have blood drawn for routine HIV viral load (VL) analysis at every study visit as part of their routine medical care. There were no restrictions on the co-existence of comorbidities for enrollment. Prospective enrollment of participants occurred on a first-come and first-served basis during their clinic visit. After obtaining informed consent, 4–6 mL of whole blood were collected in 1 ethylenediaminetetraacetic acid tube from peripheral venipuncture during the participant's clinical blood draw. A maximum of 3 study visits (ie, 3 blood samples) were obtained within a 48-week period of time. Participants were compensated $10.00 for every study visit, for a maximum of $30.00. Study visits were dependent on participants' scheduled follow-up with their provider, but were required to be at least 2 weeks apart to allow for 1 half-life of TFV-DP in DBS [11]. Study enrollment was initiated in June 2014, and follow-up for the last enrolled participant concluded in July 2017. Throughout this period, the rate of viral suppression within the University of Colorado Hospital Infectious Diseases Group Practice ranged from 84–90%, and TDF continued to be the most-prescribed nucleoside analogue, as the clinic's prescribing practices did not automatically switch to TAF after its 2015 approval. The study was approved by the Colorado Multiple Institutional Review Board (COMIRB #13–2104) and registered with clinicaltrials.gov (NCT02012621).

Our primary outcome, HIV VL, was evaluated at all study visits to inform patient care, and DBS samples were collected to coincide with these available measurements. Through August 2016, DBS at each study visit were consecutively assayed, regardless of HIV VL, as originally planned. At this point, logistical and practical constraints limited our analytical capacity and a reduced DBS assay strategy was implemented. For the remainder of the study, DBS assays were performed for all study visits for participants with a detectable HIV VL measurement at 1 or more visits, while DBS assays were discontinued for participants with suppressed HIV VL at all observed visits. This outcome-dependent sampling strategy is similar to a case (viremic) control (suppressed) study design extended to a longitudinal setting. Under outcome-dependent sampling, statistical power is maintained by enriching the sample for the reasonably rare outcome (<20% of study visits) [24].

Quantification of Tenofovir Diphosphate in Dried Blood Spots

For the DBS preparation, 25 mcl of whole blood were pipetted 5 times into a Whatman 903 Protein Saver card, as previously described [11, 12]. These cards were allowed to dry at room temperature for at least 2 hrs (and up to overnight), after which they were stored in plastic bags with humidity indicators at -80°C until analysis [11, 12, 14]. TFV-DP was quantified from a 3 mm punch using liquid chromatography/tandem mass spectrometry, as previously validated [12].

Self-reported Adherence Assessment

At each study visit, participants were asked about their 3-month adherence to their current ART regimen using a visual analog scale ranging from 0% to 100% adherence [25, 26]. Briefly, study personnel asked the participant to mark on a horizontal line that ranged from 0% to 100%, with predefined intervals every 10%, their best estimate of adherence in that period. Since study visits were dependent on scheduling for clinical care, overlap on the time period assessing self-reported adherence was possible.

HIV Viral Load Analysis

Quantitative HIV-1 VL analysis was performed using the Roche cobas 6800 HIV test (linear range 20 to 10^7 copies/mL) at the University of Colorado Hospital clinical laboratory, which is certified under the Clinical Laboratory Improvement Amendment of 1988.

Statistical Analysis

TFV-DP concentrations were log-transformed for analysis to address right skew, and results were back-transformed to the geometric mean for ease of interpretation in units of drug concentration (fmol/punch) [27]. Baseline TFV-DP concentrations were compared using *t*-tests or analyses of variance, or their non-parametric counterparts, as appropriate. In order to analyze all available visit data, drug concentrations below the limit of quantification were imputed to 12.5 fmol/punch, which is between 0 and the lower limit of quantification of the assay [12]. TFV-DP concentrations were classified into 5 categories based on previous studies in HIV-uninfected individuals [14]. While the adherence:concentration benchmarks in PLWH have not been determined, this classification was considered to be a reasonable starting point for this analysis. To accommodate repeated measures over time, generalized estimating equations with a logit link were used to estimate the odds ratios (OR) of viral suppression (<20 copies/mL), comparing each TFV-DP category to the reference category of <350 fmol/punch. An adjusted OR (aOR) was obtained by including covariates for age, gender, race, body mass index (BMI), estimated glomerular filtration rate (using Modification of Diet in Renal Disease equation), $CD4^+$ T-cell count, ART class, and duration of ART in the model. Adjustment variables were selected *a priori* based on previous findings on the pharmacology of TFV

in plasma [28, 29] and TFV-DP in peripheral blood mononuclear cells (PBMCs) [30] and DBS [14]. Current ART therapy duration was also categorized, with the highest category being >6 months, when TFV-DP in DBS would be at a steady state [11, 14]. A separate, generalized estimating equation model of viral suppression considered the association of suppression with the 3-month, self-reported adherence categories: <28.5% (reference category), 28.5% to <50%, 50% to 84%, 85% to 99%, and 100%, aiming to mirror the TFV-DP categories and also based on previous studies in HIV-uninfected volunteers. These models of viral suppression, utilizing either TFV-DP in DBS or 3-month self-reported adherence, were compared using the quasilikelihood under the independence model criterion [31].

To address the outcome-dependent sampling and confirm the strength of our findings, we performed a sensitivity analysis limiting data to those collected during the initial phase of the study (ie, before August 2016, when the DBS assay strategy was modified).

RESULTS

Study Population

A total of 807 participants were enrolled, 619 (77%) of whom were suppressed to <20 copies/mL at enrollment, contributing 1939 person-visits and 1936 DBS samples, with 444 completing all 3 visits (275 suppressed at all visits), 244 completing 2 visits (189 suppressed at both visits), and 119 completing only 1 visit (98 suppressed). The demographic characteristics of the participants for whom DBS were analyzed for drug concentrations (n = 532) are presented in Table 1, and comparisons with the participants for whom drug concentrations in DBS were not quantified (n = 272) are presented in Supplementary Table 1, without major differences observed between these 2 groups.

Among the 532 participants for whom DBS were analyzed (n = 1199 person-visits), the median time between visits was 16 (range 2, 48) weeks. Across person-visits, 839 (70%) samples had an HIV VL <20 copies/mL, 213 (18%) had 20–200, and 147 (12%) had >200 copies/mL. The HIV VL in viremic participants upon enrollment is shown in Table 1, and ranged from 20 to 331 000 copies/mL.

Tenofovir Diphosphate Concentrations in Dried Blood Spots

TFV-DP was quantified in DBS from 1199 person-visits (derived from 532 participants) and included in the drug concentration analysis; 11 samples (<1%) were below the limit of quantification. TFV-DP concentrations were available for 521 participants at enrollment (7 samples had no paired HIV VL and 4 samples had missing drug concentrations). The baseline demographic characteristics of these 521 participants are shown in Table 1. The geometric mean concentration of TFV-DP in all participants at enrollment was 1450 fmol/punch (95% confidence interval [CI] 1357–1548), with higher overall concentrations in the suppressed (1728, 95% CI 1601–1865) vs. viremic participants (1021, 95% CI 917–1138; $P < .0001$).

The TFV-DP concentrations, according to participant characteristics and viral suppression status at enrollment, are shown in Table 2. In an unadjusted analysis limited to the suppressed group only, TFV-DP concentrations were significantly lower in Blacks (1453, 95% CI 1291–1635) vs. Whites (1793, 95% CI 1678–1916; $P = .002$) and Hispanics (1760, 95% CI 1563–1982; $P = .025$). In this same group, participants with BMIs <18.5 Kg/m^2 had higher TFV-DP concentrations (2404, 95% CI 1875–3082) when compared to participants with a BMI 25–30 Kg/m^2 (1718, 95% CI 1577–1870; $P = .012$) or >30 Kg/m^2 (1416, 95% CI 1271–1578; $P = .0002$). Furthermore, suppressed participants taking non-nucleoside reverse transcription inhibitor (NNRTI)-based regimens also had lower concentrations of TFV-DP (1563, 95% CI 1432–1707) when compared to participants taking a boosted protease inhibitor (1890, 95% CI 1704–2095; $P = .006$) or a multiclass regimen (1927, 95% CI 1650–2252; $P = .022$). Lastly, use of a pharmacologic booster among virologically-suppressed participants (1888, 95% CI 1749–2037) was associated with higher TFV-DP concentrations compared to no booster use (1610, 95% CI 1505–1723; $P = .002$).

In both viremic and suppressed participants, a longer current ART duration upon enrollment was associated with higher TFV-DP concentrations (Table 2). Similarly, drug concentrations increased significantly with higher 3-month, self-reported adherence in both groups, as shown in Table 2. TFV-DP concentrations in viremic and suppressed participants who reported 100% adherence were 1766 (95% CI 1313–2375) and 1863 (95% CI 1726–2010) fmol/punch, respectively.

After adjusting for age, race, gender, BMI, CD4$^+$ T-cell count, estimated glomerular filtration rate, ART class, duration of therapy, and self-reported adherence in the virologically-suppressed group, male participants had 13% (95% CI -24–-0.8; $P = .037$) lower TFV-DP concentrations compared to women (Supplementary Table 2). Similarly, Whites and Hispanics had 15% (95% CI 0.4–31; $P = .044$) and 20% (95% CI 2–41; $P = .024$) higher TFV-DP concentrations vs Blacks, respectively. Participants with BMIs >30 Kg/m^2 had 47% (95% CI 31–59; $P < .0001$) lower drug concentrations compared to those with BMIs <18.5 Kg/m^2 (Supplementary Table 2).

Self-reported Adherence

A total of 482 participants (337 suppressed, 145 viremic) had data available for 3-month, self-reported adherence at enrollment, with a median adherence of 99% (interquartile range [IQR] 90–100) and 90% (IQR 80–100) adherence in suppressed and viremic participants, respectively (Table 1). Median adherence was similar in women (100%, IQR 90–100) vs men (98%, IQR 90–100; $P = .38$) and in Blacks (95%, IQR 90–100) compared to Whites (98%, IQR 90–100; $P = .18$) and Hispanics (98%, IQR 90–100; $P = .65$). Adherence in participants taking an NNRTI-based regimen (100%, IQR 94–100) was higher compared to participants taking a boosted protease inhibitor–based (96%,

Table 1. Demographic Characteristics of Enrolled Participants Included in the Analysis

		Participants With DBS at Enrollment Visit (n = 521)	
	Participants Included in Analysis (n = 532)	No. (%) or Median (IQR)	
Characteristic	No. (%) or Median (IQR)	Viremic (n = 174)	Suppressed (n = 347)
Age	46 (36, 52)	43 (33, 51)	46 (38, 53)
Gender			
Female	76 (14%)	18 (10%)	56 (16%)
Male	456 (86%)	156 (90%)	291 (84%)
Race/ethnicity			
Black	101 (19%)	35 (20%)	64 (18%)
White	305 (57%)	95 (55%)	203 (59%)
Hispanic	101 (19%)	36 (21%)	63 (18%)
Other	25 (5%)	8 (5%)	17 (5%)
Body mass index (Kg/m^2)			
<18.5	21 (4%)	7 (4%)	14 (4%)
18.5–25	225 (42%)	80 (46%)	138 (40%)
25–30	175 (33%)	54 (31%)	119 (34%)
>30	109 (21%)	33 (19%)	74 (21%)
eGFR (mL/min)	87 (74, 102)	90 (76, 105)	86 (73, 100)
CD4$^+$ T-cell count (cells/mm^3)			
<200	58 (11%)	43 (25%)	15 (4%)
200–350	80 (15%)	29 (17%)	49 (14%)
350–500	78 (15%)	26 (15%)	49 (14%)
>500	316 (59%)	76 (44%)	234 (67%)
HIV viral load (copies/mL)	132 (43, 699)	132 (42, 660)	-
Time on current ART (months)			
<1	21 (4%)	17 (10%)	4 (1%)
1–3	45 (8%)	22 (13%)	22 (6%)
3–6	30 (6%)	15 (9%)	15 (4%)
>6	436 (82%)	120 (69%)	306 (88%)
Type of ART			
NNRTI-based	141 (27%)	22 (13%)	116 (33%)
INSTI-based	191 (36%)	77 (44%)	110 (32%)
b/PI-based	133 (25%)	47 (27%)	84 (24%)
Multiclass	67 (13%)	28 (16%)	37 (11%)
Pharmacologic booster			
No	259 (49%)	60 (34%)	193 (56%)
Yes	273 (51%)	114 (66%)	154 (44%)
3-month self-reported adherence (%)[a]	98 (90, 100)	90 (80, 100)	99 (90, 100)

Abbreviations: ART, antiretroviral therapy; b/PI, boosted protease inhibitor; DBS, dried blood spots; eGFR, estimated glomerular filtration rate; HIV, human immunodeficiency virus; INSTI: integrase strand-transfer inhibitor; IQR, inter-quartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor.

[a]Data on self-reported adherence in the preceding 3 months were available for n = 482 participants.

IQR 85–100; P = .01), an integrase-based (97%, IQR 90–100; P = .07), or a multiclass-based (95%, IQR 87–100; P = .006) regimen. Participants taking a pharmacologic booster (95%, IQR 85–100) had lower adherence compared to those who were not (99%, IQR 93–100; P < .0001).

Association of Tenofovir Diphosphate in Dried Blood Spots With Viral Suppression

The distribution of all person-visits by TFV-DP concentration category and 3-month self-reported adherence are shown in Tables 3 and 4, respectively. The aORs of viral suppression, based on the TFV-DP concentration categories, are shown in Table 3 and Figure 1. The highest estimated aOR for suppression was 73.5 (95% CI 25.7–210.5; P < .0001) for TFV-DP concentrations ≥1850 fmol/punch vs <350 fmol/punch. The estimated aOR of viral suppression, based on self-reported adherence, are shown in Table 4 and Supplementary Figure 1, estimated as 8.5 (95% CI 2.3–30.9; P = .0012) for 100% vs. <28.5% adherence and 8.6 (95% CI 2.3–31.2; P = .011) for 85–99% vs. <28.5% adherence. The quasilikelihood under the independence model criterion for the model including TFV-DP in DBS was substantially lower vs. the model including 3-month self-reported adherence (1149 vs. 1233), indicating the TFV-DP model fit the data better.

Table 2. TFV-DP Concentrations in Dried Blood Spots at the Time of Enrollment

	TFV-DP (fmol/punch) GM (95% CI)				
Characteristic	All Participants (n = 521)	Viremic (n = 174)	*P*-Value	Suppressed (n = 347)	*P*-Value
Gender					
Male	1436 (1338, 1542)	1045 (885, 1233)	REF	1704 (1611, 1802)	REF
Female	1533 (1287, 1826)	842 (516, 1373)	.41	1860 (1637, 2112)	.22
Race/ethnicity					
Black	1253 (1077, 1457)	955 (671, 1359)	REF	1453 (1291, 1635)	REF
White	1508 (1382,1645)	1041 (841, 1290)	.680	1793 (1678, 1916)	*.002*
Hispanic	1479 (1272, 1720)	1092 (771,1546)	.600	1760 (1563, 1982)	*.025*
Other	1493 (1106, 2016)	808 (386, 1691)	.690	1993 (1585, 2506)	*.016*
Body mass index (Kg/m^2)					
<18.5	1750 (1260, 2430)	928 (421, 2043)	REF	2404 (1875, 3082)	REF
18.5–25	1465 (1324, 1623)	968 (767, 1223)	.920	1863 (1722, 2017)	.056
25–30	1494 (1333, 1675)	1099 (827, 1460)	.690	1718 (1577, 1870)	*.012*
>30	1293 (1118, 1495)	1054 (732, 1516)	.770	1416 (1271, 1578)	*.0002*
CD4$^+$ T-cell count (cells/mm^3)					
<200	988 (814, 1200)	809 (590, 1109)	REF	1754 (1369, 2246)	REF
200–350	1402 (1185, 1657)	1036 (705, 1521)	.328	1676 (1462, 1922)	.750
350–500	1381 (1164, 1638)	952 (634, 1428)	.534	1682 (1467, 1929)	.770
>500	1589 (1461, 1729)	1188 (937, 1506)	.056	1747 (1641, 1860)	.980
HIV viral load (copies/mL)					
<20	1728 (1608, 1857)	…	…	1728 (1608, 1857)	…
20–200	1469 (1283, 1681)	1469 (1283, 1681)	…	…	…
>200	633 (542, 739)	633 (542, 739)	…	…	…
Time on current ART (months)					
<1	691 (501 954)	660 (402, 1084)	REF	842 (525, 1351)	REF
1–3	1523 (1219, 1902)	1389 (898, 2148)	*.028*	1669 (1365, 2042)	*.009*
3–6	1754 (1340, 2297)	1567 (924, 2658)	*.020*	1964 (1539, 2507)	*.002*
>6	1476 (1374, 1585)	974 (808, 1174)	.150	1738 (1646, 1834)	*.003*
Type of ART					
NNRTI-based	1428 (1256, 1623)	885 (567, 1380)	REF	1563 (1432, 1707)	REF
INSTI-based	1433 (1283, 1599)	1095 (863, 1389)	.400	1729 (1580, 1892)	.117
b/PI-based	1518 (1330, 1731)	1026 (757, 1391)	.590	1890 (1704, 2095)	*.006*
Multiclass	1413 (1172, 1704)	938 (632, 1391)	.850	1927 (1650, 2252)	*.022*
Pharmacologic booster					
No	1431 (1302, 1573)	979 (748, 1280)	REF	1610 (1505, 1723)	REF
Yes	1468 (1339, 1609)	1045, (860, 1269)	.700	1888 (1749, 2037)	*.002*
3-month self-reported adherence (%)					
100	1840 (1665, 2035)	1766 (1312, 2375)	REF	1863 (1726, 2010)	REF
85 to 99	1519 (1377, 1676)	1144 (877, 1491)	*.032*	1686 (1560, 1821)	*.071*
50 to 84	802 (673, 955)	456 (326, 639)	*<.0001*	1488 (1256, 1763)	*.018*
28 to <50	545 (356, 833)	475 (247, 916)	*.0004*	1002 (514, 1954)	*.071*
<28.5[a]	680 (336, 1375)	561 (180, 1749)	*.056*	1207 (470, 3102)	…

The italicized values are meant to show statistical significance.

Abbreviations: ART, antiretroviral therapy; b/PI, boosted protease inhibitor; CI, confidence interval; GM, geometric mean; HIV, human immunodeficiency virus; INSTI, integrase strand-transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; REF, reference; TFV-DP, tenofovir diphosphate.

[a]Only 1 participant who was suppressed reported <28.5% adherence.

Sensitivity Analysis

In the subset of participants for whom DBS were consecutively analyzed prior to the modification of the assay strategy, the aOR for viral suppression was 81.3 (95% CI 17.9–368.6; $P < .0001$) for a TFV-DP concentration of ≥1850 fmol/punch, compared to <350 fmol/punch (Supplementary Table 3).

DISCUSSION

In this study, we found that TFV-DP in DBS was strongly associated with virologic suppression to <20 copies/mL, and that this association was stronger than self-reported adherence. This association increased with higher TFV-DP concentrations, and remained significant after covariate adjustment, including ART class and therapy duration. In comparison to the extensive body

Table 3. Odds Ratio of Viral Suppression by TFV-DP in Dried Blood Spots (n = 1199 Person-Visits)

TFV-DP (fmol/punch)	Person-Visits, n (%)	Median (IQR) HIV VL, copies/mL	OR Suppression (95% CI)	*P*-Value	aOR Suppression[a] (95% CI)	*P*-Value
<350	47 (4%)	17 540 (3600, 51 300)	1	REF	1	REF
351 to 699	63 (5%)	312 (70, 7640)	5.7 (2.3, 14.4)	*.0002*	8.9 (2.6, 30.4)	*.0005*
700 to 1249	217 (18%)	142 (38, 1268)	18.1 (8.4, 39.0)	*<.0001*	32.8 (11.6, 93.1)	*<.0001*
≥1250 to 1849	357 (30%)	51 (28, 146)	31.2 (14.5, 67.5)	*<.0001*	49.2, (17.3, 139.8)	*<.0001*
≥1850	515 (43%)	55 (33, 219)	40.3 (18.7, 87.1)	*<.0001*	73.5 (25.7, 210.5)	*<.0001*

The italicized values are meant to show statistical significance.

Abbreviations: aOR, adjusted odds ratio; ART, antiretroviral therapy; CI, confidence interval; HIV VL, human immunodeficiency virus viral load; IQR, interquartile range; OR, odds ratio; TFV-DP, tenofovir diphosphate;

[a]Adjusted for age, gender, race, body mass index, estimated glomerular filtration rate, $CD4^+$ T-cell count, ART class, and duration of ART.

of literature that has demonstrated the utility of TFV-DP in DBS as a predictor of PrEP efficacy [15–17], this study provides new additional insights about its association with viral suppression and potential applications in HIV infection.

Our findings suggest PLWH may have higher TFV-DP concentrations in DBS than HIV-uninfected volunteers [11, 14] and that TFV-DP concentrations in DBS are likely to be influenced by individual characteristics unique to this population, such as the use of concomitant medications or additional comorbidities. For example, while TFV-DP concentrations were lower in virologically-suppressed Black PLWH compared to other races, they were still higher than previous estimates in HIV-uninfected Blacks [14], despite similar self-reported adherence. This suggests that ART adherence may not be the main driver of these differences; they could be influenced by drug-drug interactions, chronic inflammation [32], and/or unique pharmacogenetics/biology in PLWH. Similarly, BMI was also associated with TFV-DP concentrations in participants with viral suppression, even after adjusting for race and gender, suggesting that TFV and TFV-DP exposure could be influenced by body habitus. This is consistent with previous observations, where low BMI was associated with high plasma TFV [28, 29, 33]. We also observed associations between TFV-DP exposure and ART regimen. For instance, virally-suppressed participants taking an NNRTI-based regimen (mainly efavirenz) had an average TFV-DP concentration of 1563 fmol/punch vs. 1888 fmol/punch for suppressed participants taking a regimen including a pharmacologic booster. These findings are consistent with previous observations, where high levels of TFV in plasma [28–30, 34–38], TFV-DP in PBMCs [30, 39] and TFV in hair [40] were observed in patients taking pharmacologic boosters. Among the possible explanations are that ritonavir and cobicistat may increase TDF (and consequently TFV) uptake into the systemic circulation by altering the activity of P-glycoprotein (ie, via reduction of TDF efflux) and by inhibiting esterase cleavage of TDF at the luminal gut level [41] or that pharmacologic boosters may slow the renal clearance of TFV due to an inhibition of efflux transporters, such as the multidrug resistance proteins 4 and 2 in the renal proximal tubule [38, 42]. Collectively, these findings suggest TFV-DP in DBS may have a different pharmacokinetic profile in PLWH compared to HIV-uninfected adults, and that the known adherence benchmarks in the HIV-uninfected population are unlikely to apply directly to PLWH. A directly-observed dosing study in PLWH would help establish expected TFV-DP benchmarks in this population and help parse sources of TFV-DP variability.

Along with the association of TFV-DP with viral suppression in PLWH, potential applications in clinical practice should be discussed. These include the possibility of utilizing TFV-DP in DBS as a tool to inform ART adherence and exposure beyond HIV VL, in particular if this biomarker is developed as a point-of-care test. While HIV VL has been traditionally used as a surrogate for ART adherence, it cannot provide information about drug exposure or pharmacologic forgiveness. For example, HIV

Table 4. OR of Viral Suppression by Self-reported Adherence in the Preceding 3 Months (n = 1152 Person-Visits)

3-Month Self-report (%)	Person-Visits, n (%)	Median (IQR) HIV VL (copies/mL)	OR Suppression (95% CI)	*P*-Value	aOR Suppression[a] (95% CI)	*P*-Value
<28.5	10 (1%)	49 700 (3210, 57 900)	1	REF	1	REF
28 to <50	18 (2%)	474 (120, 27 775)	1.5 (0.4, 5.4)	.55	1.6 (0.3, 8.9)	.57
50 to 84	137 (12%)	624 (85, 24 150)	3.4 (1.3, 8.8)	*.0135*	3.0 (0.8, 11.4)	.11
85% to 99	456 (40%)	64 (36, 340)	8.9 (3.5, 22.5)	*<.0001*	8.6 (2.3, 31.2)	*.0011*
100	531 (46%)	53 (30, 298)	10.3 (4.1, 26.1)	*<.0001*	8.5 (2.3, 30.9)	*.0012*

The italicized values are meant to show statistical significance.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HIV VL, human immunodeficiency virus viral load; IQR, interquartile range; OR, odds ratio.

[a]Adjusted for age, gender, race, body mass index, estimated glomerular filtration rate, $CD4^+$ T-cell count, ART class and duration of ART.

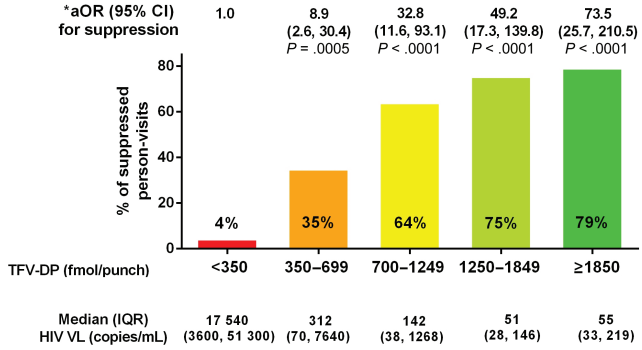

Figure 1. The aOR of HIV VL <20 copies/mL by concentration of TFV-DP in dried blood spots (N = 1199 person visits). Abbreviations: aOR, adjusted odds ratio; ART, antiretroviral therapy; CI, confidence interval; HIV VL, human immunodeficiency virus virus load; IQR, interquartile range; TFV-DP, tenofovir diphosphate. *Adjusted for age, gender, race, body mass index, estimated glomerular filtration rate, CD4+ T-cell count, ART class, and duration of ART.

VL cannot detect low drug exposure in suppressed individuals, which could trigger an early intervention to improve adherence and prevent viral rebound. In this context, TFV-DP in DBS could serve as a true predictor of viral rebound, in particular if a low concentration is associated with future viremia (despite viral suppression at the time of analysis). Conversely, since HIV VL cannot identify high cumulative drug exposure in the presence of viremia, a high TFV-DP concentration in a patient with a persistently-elevated HIV VL could suggest drug resistance and trigger early HIV genotyping. Furthermore, given its unique pharmacology, TFV-DP in DBS could also be used as a tool to monitor and/or predict drug-related toxicity in clinical practice, as it is plausible that high accumulation could precede overt clinical toxicity or drug discontinuation (as seen in high-risk individuals taking TDF for PrEP) [43, 44]. Future research is needed to evaluate these potential applications.

Our study offers several strengths, including a large sample size within a prospective clinical cohort reflective of a routine clinical practice. This provides for generalizability of our findings and sets the framework for the use of TFV-DP as a measure of cumulative adherence and exposure in clinical care. Additional strengths include the stronger association of this objective adherence biomarker in comparison with self-reported ART adherence and the range of potential applications in clinical practice. Among the limitations are that our study was observational, with adherence based on self-report and virologic suppression. In addition, our outcome-dependent sampling strategy could have strengthened our association, although our sensitivity analysis, limited to person-visits that were consecutively obtained, provided a similar, although larger, aOR. Lastly, the study was restricted to participants on TDF-based regimens. However, DBS from participants switching to TAF-based regimens during the course of the study were collected and will be analyzed in the future.

In summary, we demonstrated that TFV-DP in DBS is strongly associated with viral suppression in PLWH. In addition, TFV-DP exposure in our population was higher than that previously observed in HIV-negative volunteers, and was associated with several patient characteristics, such as race, BMI, ART class, and the use of a pharmacologic booster. Further research is needed to better understand how these individual factors contribute to the pharmacology of TFV-DP in patients on ART, including those on TDF- and TAF-based regimens. Finally, the utility of this adherence biomarker as a tool to monitor ART adherence in clinical practice should be evaluated.

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. J. R. C.-M. led the conception and study design; obtained the funding and regulatory approvals; led all aspects regarding study monitoring, logistics, data and sample collection, and result interpretation; wrote the first manuscript draft; and performed all the edits for all the subsequent drafts. M. M. performed data and statistical analysis and interpretation, generated figures and tables, and made substantial edits and critical revisions of the manuscript. R. P. C. and S. S. C. gathered participant consent and performed data and sample collection, data management, and data analysis and interpretation, and made substantial edits to and critical revisions of the manuscript. E. M. G. participated in the study design, assisted with the adherence data interpretation, and performed manuscript editing and critical revisions. J.-H. Z., L. E., and L. R. B. led the sample processing, pharmacologic analysis, and data validation for the drug concentrations and made substantial edits and critical revisions of the manuscript. J. J. K. participated in the study design, adherence, and pharmacologic data interpretation and performed manuscript editing and critical revisions. S. M. contributed to the study design and conceptualization; performed the sample size calculation, data management, statistical analysis, and interpretation; generated figures and tables; and made substantial edits and critical revisions of the manuscript. P. L. A. co-led the study conception and design, assisted with obtaining the funding, supported the study monitoring and logistics, directed and supported all aspects of the pharmacologic and drug concentration analysis, collaborated with data interpretation, and made substantial edits and critical revisions of the original manuscript and all its subsequent versions.

Acknowledgments. The authors thank the study participants and the personnel at the Colorado Antiviral Pharmacology Laboratory for their invaluable assistance and support of this study. They thank the director of the University of Colorado Hospital Human Immunodeficiency Virus program (Steven Johnson, MD) and the medical assistants (Nancy Olague, Brittany Limon, Ariel Cates, Maureen Sullivan and Missy Sorrell) at the University of Colorado Hospital-Infectious Disease Group Practice for their invaluable contributions and support of this study.

Financial support. This work was supported by the National Institutes of Health (grant numbers K23 AI104315 and R21 AI124859 to J. R. C.-M. and R01 AI122298 to P. L. A.).

Potential conflicts of interest. P. L. A. and J. J. K. received research funding from Gilead Sciences, paid to their institution. 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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