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BRIEF REPORT

Low-Level Viremia Is Associated With Cumulative Adherence to Antiretroviral Therapy in Persons With HIV

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The drivers of low-level viremia (LLV) between 20 and 200 copies/mL remain unclear. In 1042 person-visits from 497 persons with HIV on tenofovir disoproxil fumarate–containing antiretroviral therapy (ART), the association between LLV and cumulative antiretroviral adherence (quantified using tenofovir diphosphate [TFV-DP] in dried blood spots) was assessed. Lower TFV-DP levels were associated with higher odds of LLV. As TFV-DP (fmol/punch) categories decreased from >1650 to 800–1650; 800–1650 to <800; and >1650 to <800, the adjusted odds ratios for LLV vs HIV VL <20 copies/mL were 2.0 (95% CI, 1.2–3.1), 2.4 (95% CI, 1.1–5.0), and 4.6 (95% CI, 2.2–9.9), respectively. This suggests that adherence could impact LLV.

Keywords. low-level viremia; adherence; dried blood spots; tenofovir diphosphate; antiretroviral therapy.

Achieving and sustaining an undetectable HIV viral load (VL) while on antiretroviral therapy (ART) can prevent progression to AIDS, development of drug resistance, and transmission [1, 2]. Durable viral suppression is accomplished with sustained ART adherence in the majority of persons with HIV (PWH). However, some PWH develop intermittent (ie, viral blips) or persistent low-level viremia (LLV), which is usually defined as an HIV VL that is above the lower limit of detection of a specific assay (ie, <20 or <50 copies/mL) but below the defined threshold for virologic failure of 200 copies/mL [1], or even 1000 copies/mL in some settings [3, 4]. This is not an uncommon finding in clinical practice, with estimates of up to 30% of PWH on ART experiencing LLV [4]. To date, the implications of LLV remain controversial, with some studies demonstrating an association with virologic failure [5] and, in cases of persistent LLV, drug resistance [6], while other studies show no adverse clinical outcomes in cases of intermittent viral blips [7].

In addition to its uncertain clinical significance, the root causes of LLV in PWH on ART also remain unclear. They include ongoing reactivation or budding of viral reservoirs [8], drug resistance [4], or even laboratory errors. Whether ART adherence influences LLV has also been evaluated, with some studies identifying an association with adherence measures such as unannounced pill counts [9] or medication event monitoring systems (MEMS) [10], while other studies did not find an association with a combination of pill count, self-report, and MEMS [11], or with low plasma drug concentrations (which can only inform recent dosing) [12]. However, no studies have evaluated whether LLV is associated with quantitative measures of cumulative ART adherence such as tenofovir diphosphate (TFV-DP) in dried blood spots (DBS), which was the aim of this study.

METHODS

We enrolled a prospective clinical cohort of adult (≥18 years) PWH receiving any tenofovir disoproxil fumarate–based regimen at the University of Colorado Hospital (UCH) between 2014 and 2017, as previously described [13]. Study participants were recruited at the time of a routine clinical visit where blood for HIV VL was being collected and had up to 3 visits (at least 14 days apart) within a 48-week period [13]. As all the study visits were performed at the time of a regular clinic visit, the first visit upon entry to the study did not have any specific HIV viral load requirement (ie, participants were not required to have an HIV VL of <20 copies/mL at entry). After signed informed consent, 4–6 mL of whole blood in EDTA was collected for DBS and prepared by spotting 25 mcl onto 903 Protein Saver cards; DBS samples were allowed to dry for at least 2 hours and stored at −80°C until analysis [13]. Quantification of TFV-DP in DBS was performed from a 3-mm punch using a liquid chromatography/tandem mass spectrometry assay previously validated by our group [14, 15]. HIV VL in plasma was quantified using the Roche cobas 6800 HIV test at the UCH clinical laboratory, which is certified under the 1988 Clinical Laboratory Improvement Amendment (CLIA) [13]. Self-reported adherence was quantified using a validated visual analog scale, as previously reported in the cohort [13, 16]. The study was approved

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by the Colorado Multiple Institutional Review Board (COMIRB #13-2104) before the initiation of any study procedures.

To minimize the influence of recent initiation of ART on viral suppression, we only included study participants who had been on ART for at least 6 months at enrollment. For this analysis, person-visits from study participants were categorized into 1 of the following categories: (a) HIV VL <20 copies/mL (reference category); (b) HIV VL ≥20–<200 copies/mL; (c) HIV VL ≥200–<1000 copies/mL; and (d) HIV VL ≥1000 copies/mL. TFV-DP in DBS was categorized based on our previous observations of its predictive value for future viremia, as follows: (a) ≥1650; (b) 800–1650; and (c) <800 fmol/punch [17]. A generalized linear mixed model with a multinomial logistic link was used to estimate the odds ratio (ORs) for each HIV VL category compared with <20 copies/mL by comparing the 2 lowest drug concentration categories (<800 and 800–1650 fmol/punch) with the highest category (≥1650 fmol/punch), which was considered the reference. We selected a mixed model in order to include all available data as participants provided repeated measures (ie, up to 3 in 48 weeks) and could change TFV-DP categories throughout the study. As our model was highly parameterized, ORs were adjusted (aORs) for covariates that remained significant using backward selection and were not explained by TFV-DP category [13, 17]. Data are number (%), median (interquartile range [IQR]), or aOR (95% CI), and a *P* value <.05 was considered statistically significant. Statistical analyses were performed using SAS, version 9.4 (SAS Institute, Inc., Cary, NC, USA), and R software, version 3.4.4.

RESULTS

From a total of 532 study participants (1199 person-visits) in whom drug concentrations were available [13], this analysis included 497 participants (1042 person-visits) in whom all clinical covariates for an adjusted analysis were available. The demographic characteristics of the study participants (at the first visit where drug concentrations were available) according to each HIV VL category are presented in Table 1. Overall, the median (IQR) age was 46 (37–52) years, and 72 (14%) of the study participants were female. The racial distribution was consistent with the demographics of the HIV epidemic in Colorado (Table 1), as previously reported in this cohort [13]. The proportion of study participants with $CD4^+$ T cells <200 cells/mm^3 was the lowest (6%) for participants with HIV VL <20 copies/mL and highest (39%) for participants with HIV VL >1000 copies/mL (Table 1). Among participants with an HIV VL >1000 copies/mL, boosted protease inhibitor– and integrase strand transfer inhibitor (INSTI)–based ART were predominant (Table 1). Overall, 50% of participants were in the highest TFV-DP category, and the proportion of participants in this category decreased from 56% in the <20 copies/mL HIV VL category to 3% in the >1000 copies/mL HIV VL category (Table 1). Figure 1 shows this same relationship for all person-visits, where in the highest TFV-DP category the proportion of person-visits where the participants had an HIV VL <20 copies/mL was 83% compared with 1% for an HIV VL >1000 copies/mL.

Table 2 shows the aOR for each HIV VL category according to a change in each TFV-DP category. When compared with the reference HIV VL category (<20 copies/mL), the aOR for an HIV VL ≥20–<200 copies/mL increased by 2.0 (95% CI, 1.2–3.1; *P* = .0048) and by 2.4 (95% CI, 1.1–5.0; *P* = .034) for a decrease in 1 category of TFV-DP from ≥1650 fmol/punch to 800–1650 punch or from 800–1650 fmol/punch to <800 fmol/punch, respectively (Table 2). Comparatively, the aOR for an HIV VL of ≥20–<200 copies/mL increased by 4.6 (95% CI, 2.2–9.9; *P* < .0001) for a decrease in 2 categories of TFV-DP from ≥1650 to <800 fmol/punch (Table 2). We observed similar trends, albeit with a higher magnitude, for HIV VL categories of ≥200–<1000 copies/mL (Table 2) and ≥1000 copies/mL (data not shown). When we limited our analysis to visits where HIV VL was always <200 copies/mL, our results were almost identical to those observed in the full cohort (Supplementary Table 1). Similarly, in an analysis that included self-reported adherence in the last 3 months in the model, the odds of being in each HIV VL category were similar to the original model, although slightly attenuated (Supplementary Table 2). Last, we found a statistically significant difference when we compared median (IQR) concentrations of TFV-DP in DBS from person-visits where the HIV VL was <20 copies/mL with those where it was ≥20–<200 copies/mL (1839 [1390–2521] vs 1580 [1153–2261] fmol/punch; *P* = .001) (Supplementary Figure 1).

We also evaluated the association between $CD4^+$ T cells and LLV. In this analysis, adjusted by TFV-DP category and ART class, the odds of being in each HIV VL for every decrease in $CD4^+$ T cells of 100 cells/mm^3 were 1.1 (95% CI, 1.0–1.2; *P* = .031), 1.1 (95% CI, 0.9–1.3; *P* = .064), and 2.2 (95% CI, 1.4–3.4; *P* = .0006), respectively, for an HIV VL of ≥20–<200 copies/mL, ≥200–<1000 copies/mL, and >1000 copies/mL when compared with <20 copies/mL.

DISCUSSION

In this study, we established that cumulative ART adherence, quantified using TFV-DP in DBS, was associated with LLV in the ≥20–<200 copies/mL range and with higher ranges of HIV VL (ie, ≥200 and ≥1000 copies/mL). Of interest, decreasing from the highest adherence category to the middle category (≥1650 fmol/punch to 800–1650 fmol/punch) resulted in a doubling of the odds of LLV, informing pharmacologic forgiveness and sources of LLV. These results further expand our (and others') previous findings, where TFV-DP in DBS was found to be strongly associated with viral suppression [13, 18], and suggest that changes in cumulative ART adherence play a significant role in the development of LLV in PWH on ART. They

Table 1. Demographics and Clinical Characteristics of the Study Participants at Their Entry Visit According to HIV Viral Load Category

Characteristic	HIV VL, Copies/mL				Total
	<20 n = 363 769 Person-Visits	≥20–<200 n = 74 157 Person-Visits	≥200–<1000 n = 27 42 Person-Visits	≥1000 n = 33 74 Person-Visits	n = 497 1042 Person-Visits
	No. (%) or Median (IQR)				
Age, y	46 (38–53)	48 (36–54)	44 (36–48)	40 (36–51)	46 (37–52)
Gender					
Male	304 (84)	69 (93)	23 (85)	29 (88)	425 (86)
Female	59 (16)	5 (7)	4 (15)	4 (12)	72 (14)
Race					
Black	69 (19)	15 (20)	9 (33)	2 (6)	95 (19)
White	213 (59)	36 (49)	13 (48)	19 (58)	281 (57)
Hispanic	65 (18)	20 (27)	3 (11)	9 (27)	97 (20)
Other	16 (4)	3 (4)	2 (7)	3 (9)	24 (5)
BMI, kg/m^2					
<18.5	15 (4)	2 (3)	0 (0)	1 (3)	18 (4)
≥18.5–<25	149 (41)	31 (42)	13 (48)	19 (58)	212 (43)
≥25–<30	122 (34)	24 (32)	8 (30)	10 (30)	164 (33)
≥30	77 (21)	17 (23)	6 (22)	3 (9)	103 (21)
eGFR, mL/min/1.73 m^2	86 (73–101)	88 (75–104)	80 (73–99)	91 (80–109)	87 (74–102)
CD4$^+$ T-cell count, cells/mm^3					
<200	21 (6)	10 (14)	3 (11)	13 (39)	47 (9)
≥200–<350	50 (14)	11 (15)	5 (19)	5 (15)	71 (14)
≥350–<500	53 (15)	10 (14)	5 (19)	6 (18)	74 (15)
≥500	239 (66)	43 (58)	14 (52)	9 (27)	305 (61)
Hematocrit, %	45 (42–47)	45 (42–48)	45 (41–47)	43 (41–45)	45 (42–47)
Type of ART					
NNRTI-based	117 (32)	12 (16)	2 (7)	2 (6)	133 (27)
INSTI-based	121 (33)	33 (45)	7 (26)	11 (33)	172 (35)
b/PI-based	87 (24)	16 (22)	12 (44)	14 (42)	129 (26)
Multiclass	38 (10)	13 (18)	6 (22)	6 (18)	63 (13)
Pharmacologic booster					
No	200 (55)	32 (43)	6 (22)	9 (27)	247 (50)
Yes	163 (45)	42 (57)	21 (78)	24 (73)	250 (50)
TFV-DP in DBS, fmol/punch					
<800	19 (5)	11 (15)	8 (30)	25 (76)	63 (13)
800–1650	139 (38)	30 (41)	9 (33)	7 (21)	185 (37)
≥1650	205 (56)	33 (45)	10 (37)	1 (3)	249 (50)
Self-reported adherence in the last 3 mo, %	99 (90–100)	97 (90–100)	90 (68–98)	80 (60–90)	98 (90–100)

Abbreviations: ART, antiretroviral therapy; b/PI, boosted protease inhibitor; BMI, body mass index; DBS, dried blood spots; eGFR, estimated glomerular filtration rate; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; TFV-DP, tenofovir diphosphate.

also support previous observations where changes in adherence (measured by self-report or pill counts) were associated with changes in residual viral replication below the limit of detection of most clinical assays [19–21]. However, they are contrary to another study (ACTG A5321) where antiretroviral drug concentrations in hair—which also quantify cumulative ART adherence—did not find an association with single-copy viremia [22]. This discrepancy could be explained because A5321 focused on a cohort of PWH with long-standing viral suppression who had participated in ART clinical trials and did not assess HIV VL in the 20–200 range [22]. Despite these discrepancies, our findings highlight the relevance that an objective and quantitative adherence measure can have in clarifying the different thresholds of viremia that are available in clinical practice.

While previous studies have explored root causes of LLV in PWH on ART [4], to our knowledge, this is the first study that has assessed its association and potential role with a measure of cumulative adherence. This is particularly relevant because we used an objective and reproducible adherence measure that is highly informative of virologic outcomes in PWH. Furthermore, the drug concentration categories that we used in our analyses have been previously found to be predictive of future viremia, even in PWH who are virologically suppressed. In this analysis, we demonstrate that these concentration thresholds can also

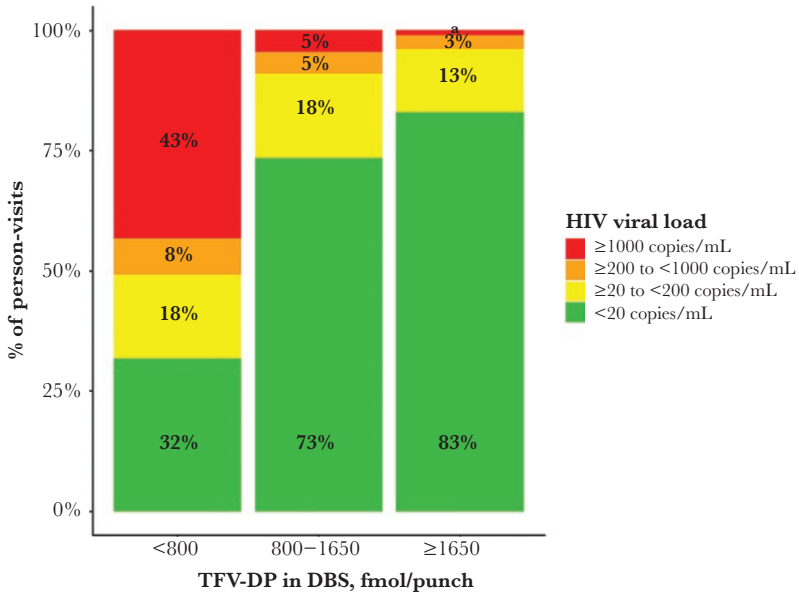

Figure 1. Proportion of person-visits according to each category of tenofovir diphosphate in dried blood spots (n = 1042 person-visits derived from 497 PWH). [a]1%. Abbreviations: DBS, dried blood spots; TFV-DP, tenofovir diphosphate.

explain changes in concomitant viremia, thus expanding the utility of TFV-DP as an adherence biomarker in PWH on ART.

In addition to the novelty of our findings, the significance of our results should be emphasized based on their potential clinical implications. First, this analysis focused on LLV, which is frequently encountered in the clinic and for which a mechanistic explanation has not yet been established. Second, it underscores the role of adherence as a modifiable factor that could be targeted to address a clinical scenario where no management consensus is currently available [1]. Third, our results identified a specific group of PWH (ie, those with HIV VL between 20 and 200 copies/mL) in which timely counseling could be implemented to prevent an adverse clinical outcome such as virologic failure. Collectively, these features support the conduct of controlled clinical trials where the use of objective pharmacologic measures of adherence to complement the information provided by routine viral load monitoring is evaluated. Such studies could prove particularly useful in PWH who are not fully virologically suppressed (ie, HIV VL 20–1000 copies/mL), in whom resistance testing is not always feasible [23].

Table 2. Adjusted Odds Ratio for Each HIV Viral Load Category Compared With <20 Copies/mL (Reference) According to Tenofovir Diphosphate in Dried Blood Spots in the Study Population (n = 1042 Person-Visits Derived From 497 PWH)

	HIV VL, Copies/mL		
	<20	≥20–<200	≥200–<1000
Decrease in TFV-DP in DBS, fmol/punch		aOR[a] (95% CI)	
From≥1650 to 800–1650	1	2.0 (1.2–3.1) *P* = .0048	2.5 (0.6–9.4) *P* = 0.16
From 800–1650 to <800	1	2.4 (1.1–5.0) *P* = 0.034	17.1 (3.5–83.6) *P* = 0.0009
From≥1650 to <800	1	4.6 (2.2–9.9) *P* < .0001	43.5 (8.2–229.0) *P* < .0001

Abbreviations: aOR, adjusted odds ratio; DBS, dried blood spots; PWH, people with HIV; TFV-DP, tenofovir diphosphate; VL, viral load.

[a]Adjusted for $CD4^+$ T-cell count and ART class; aOR for HIV VL ≥1000 copies/mL showed the same significant trends with higher magnitude in aOR, as expected, ranging from 28.5 to >999 (data not shown).

In addition, our study period (2014–2017) preceded the widespread use of TAF-based therapy and of second-generation INSTIs. However, we would anticipate similar results for PWH on TAF-based therapy. Furthermore, we limited our follow-up period to 48 weeks and lack data on the clinical outcomes (ie, virologic failure, residual inflammation) of the PWH with LLV. Finally, we did not evaluate any other potential drivers of LLV such as duration of viral suppression or disease stage at the initiation of treatment [4]; however, this was partially mediated by including only participants who had been on ART for at least 6 months. Additional studies focusing on modern ART regimens and on a more comprehensive and intensive evaluation of LLV and cumulative adherence are required.

The strengths of our study include a large sample size that was prospectively enrolled within in a diverse real-world clinical cohort of PWH taking a variety of ART regimens. This allows for wide reach and applicability of our results in different populations. We also utilized a novel objective biomarker of cumulative adherence (TFV-DP in DBS) that is highly informative of concurrent suppression and future viremia. Among our weaknesses is that we were not able to differentiate participants who had persistent low-level viremia vs a viral blip.

In conclusion, we identified an association of LLV (HIV VL between 20 and 200 copies/mL) and a decrease in cumulative ART adherence measured using TFV-DP in DBS. These

findings emphasize the possible role that treatment adherence could play on the development of LLV and identify a potential interventional target to address this common clinical scenario. Future studies should evaluate whether TFV-DP in DBS can be used to identify patients with LLV in whom an adherence intervention would be beneficial.

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Author contributions. J.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 contributed to all the edits for the subsequent drafts. M.M. performed data and statistical analysis and interpretation, generated figures and tables, and performed edits in subsequent versions. R.P.C. and S.S.C. performed participant consent, data and sample collection, data management, and data analysis and interpretation and made substantial edits and critical revisions of the manuscript. 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. 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. S.M. co-led the conception of the study design and conceptualization, performed the sample size calculation and data management, led the statistical analysis and interpretation, generated figures and tables, and made substantial edits and critical revisions of the manuscript.

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