Emtricitabine triphosphate in dried blood spots is a predictor of viral suppression in HIV infection and reflects short-term adherence to antiretroviral therapy

Katherine Frasca¹, Mary Morrow², Ryan P. Coyle¹, Stacey S. Coleman³, Lucas Ellison⁴, Lane R. Bushman⁴, Jennifer J. Kiser⁴, Jia-Hua Zheng⁴, Samantha Mawhinney², Peter L. Anderson⁴ and Jose Castillo-Mancilla^{1*}

¹Division of Infectious Diseases, School of Medicine, University of Colorado-AMC, Aurora, CO, USA; ²Department of Biostatistics and Bioinformatics, Colorado School of Public Health, Aurora, CO, USA; ³Duke University Hospital, Durham, NC, USA; ⁴Colorado Antiviral Pharmacology Laboratory and Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado-AMC, Aurora, CO, USA

*Corresponding author. Division of Infectious Diseases, Department of Medicine, University of Colorado Anschutz Medical Campus, 12700 E 19th Ave., B168, Aurora, CO 80045, USA. Tel: +1 303 724 4934; Fax: +1 303 724 4926; E-mail: jose.castillo-mancilla@ucdenver.edu

Received 6 September 2018; returned 24 October 2018; revised 28 November 2018; accepted 10 December 2018

Background: Emtricitabine triphosphate (FTC-TP), the phosphorylated anabolite of emtricitabine, can be quantified in dried blood spots (DBS). We evaluated FTC-TP in DBS as a predictor of viral suppression and evaluated self-reported adherence as a predictor of FTC-TP.

Methods: Persons living with HIV (PLWH) on an FTC-containing regimen were prospectively recruited. A DBS and HIV viral load were obtained during routine clinical visits. Self-reported adherence for 3 days, 30 days and 3 months was captured. Generalized estimating equations were used to estimate the adjusted odds ratio (aOR) of viral suppression for quantifiable FTC-TP versus below the limit of quantification (BLQ). The utility of self-reported adherence to predict quantifiable FTC-TP was assessed by calculating the area under receiver operating characteristic (ROC) curve.

Results: One thousand one hundred and fifty-four person-visits from 514 participants who had DBS assayed for FTC-TP were included in the analysis. After adjusting for age, gender, race, BMI, ART class, ART duration, estimated glomerular filtration rate and CD4+ T cell count, the aOR (95% CI) for viral suppression for quantifiable FTC-TP versus BLQ was 7.2 (4.3–12.0; $P < 0.0001$). After further adjusting for tenofovir diphosphate, the aOR was 2.1 (1.2–4.0; $P < 0.015$). The area under the ROC curve for 3 day self-reported adherence was 0.82 (95% CI 0.75–0.88) compared with 0.70 (95% CI 0.62–0.77, $P = 0.004$) and 0.79 (95% CI 0.71–0.86, $P = 0.32$) for 3 month and 30 day self-reported adherence, respectively.

Conclusions: In PLWH, FTC-TP from DBS is a strong predictor of viral suppression, even after adjusting for tenofovir diphosphate, and was best predicted by 3 day self-reported adherence.

Introduction

Adherence to ART is key for persons living with HIV (PLWH) to achieve and maintain viral suppression, to avoid progression to AIDS and to prevent HIV transmission.¹ Many methods have been described to quantify adherence among individuals on ART,^{2–4} but they face several limitations, including social desirability bias of self-reporting and inaccuracies of pill counts.⁴ Thus, new methods that can objectively quantify ART adherence are required.

Recently, new methods that reflect longer periods of adherence and exposure have been developed. Amongst them is the quantification of tenofovir diphosphate, the phosphorylated anabolite of tenofovir, in RBCs using dried blood spots (DBS).⁵ Tenofovir

diphosphate in the DBS is a measure of cumulative tenofovir exposure⁵ owing to its long intracellular half-life of 17 days.⁵ This adherence biomarker has been widely applied to quantify adherence and predict efficacy of tenofovir disoproxil fumarate/emtricitabine in HIV pre-exposure prophylaxis.^{6,7} In HIV treatment, tenofovir diphosphate was recently shown to be a strong predictor of viral suppression⁸ and to correlate with other measures of adherence, such as pharmacy refills⁹ and real-time adherence monitoring.¹⁰ However, owing to its long half-life in both RBCs and DBS, tenofovir diphosphate is best used as a marker of adherence over the preceding 6–8 weeks⁹ and cannot quantify changes in recent adherence. Comparatively, emtricitabine triphosphate (FTC-TP), the

phosphorylated anabolite of emtricitabine, can be simultaneously quantified in the same DBS sample along with tenofovir diphosphate, and reflects recent dosing owing to its shorter half-life of 35 h in both RBCs and DBS, thereby being more sensitive to recent changes in adherence.¹¹ To date, the association of FTC-TP in DBS with viral suppression and with other methods used to quantify adherence in HIV infection (such as self-reporting) has not been evaluated, which was the goal of this study.

Methods

Study design and participants

PLWH were prospectively recruited from the University of Colorado Hospital Infectious Diseases Group Practice at the time of their clinical visits. Inclusion criteria required that participants were ≥ 18 years of age, had HIV infection being treated with a tenofovir-based regimen, and were scheduled for a blood draw for routine laboratory analysis [which included HIV viral load (VL)]. The only exclusion criterion was pregnancy, owing to the unknown pharmacokinetics of FTC-TP in DBS in pregnant women. Permission was obtained from the participant's primary care provider prior to study personnel having any contact with the participants. If participants were interested in the study, they met with the study personnel and underwent the process of informed consent prior to sample collection. After written consent was obtained, 4–6 mL of whole blood was collected in an EDTA tube via the same venipuncture used during the participant's routine laboratory draw. Additional samples were obtained at the participant's next clinic visit, whenever blood for an HIV VL was drawn by the clinical provider, for a total of up to three visits in a 48 week period (with a minimum time of 14 days between visits to allow for one half-life of tenofovir diphosphate⁵).

Ethics

This study was approved by the Colorado Multiple Institutional Review Board (COMIRB#13-2104) and registered at clinicaltrials.gov (NCT02012621). Study enrolment was initiated in June 2014, and follow-up for the last enrolled participant was concluded in July 2017. All participants completed informed consent prior to participation in the study.

Quantification of FTC-TP and tenofovir diphosphate in DBS

After collection, 25 μ L of whole blood from the EDTA tube was spotted onto each circle of a Whatman 903 Protein Saver card and allowed to dry for at least 3 h (up to overnight). Once dry, DBS cards were stored at -80°C in plastic bags with desiccant and humidity indicators until analysis. FTC-TP and tenofovir diphosphate, for which stability in stored frozen DBS samples at -80°C has been demonstrated, were quantified from a 3 mm punch using LC–MS/MS, as previously reported.^{5,12}

Self-reported adherence

At each study visit, the participants were asked about their 3 month, 30 day and 3 day adherence to their current ART using a visual analogue scale that ranged from 0% to 100% adherence, with intervals at 10% increments, as previously described.^{8,13,14} Participants were asked to draw a mark on the scale indicating their estimated percentage of ART taken over the preceding period of time. If a mark was placed between two intervals (i.e. between 60% and 70%), the participants were asked to estimate a precise percentage of ART taken over that time frame.

HIV viral load

HIV-1 VL was quantified at the University of Colorado Hospital clinical laboratory using the Roche COBAS 6800 HIV test (range of quantification 20– 10^7 copies/mL). This laboratory is certified by the Clinical Laboratory Improvement Amendment of 1988 (CLIA). An undetectable HIV VL was therefore defined as <20 copies/mL.

Statistical analysis

FTC-TP in DBS at each person-visit was categorized as quantifiable or below the limit of quantification (BLQ, 0.1 pmol/sample), as previously described.¹¹ Tenofovir diphosphate in DBS at each person-visit was categorized based on previous data in HIV-negative persons and PLWH.^{8,15} Self-reported adherence was categorized as $<50\%$, 50%–84%, 85%–99% and 100%, based on previous clinically significant thresholds.^{16–20}

The primary outcome for the study was HIV VL, which was evaluated in all study visits as part of routine patient care. As mentioned, DBS samples were simultaneously collected along with every HIV VL assessment. From study initiation (June 2014) to August 2016, all DBS samples were consecutively analysed independently of the HIV VL result (as originally planned). However, our capacity to analyse all collected DBS within the entire cohort was limited by practical and logistical constraints. Thus, after August 2016 the assay strategy was modified, focusing only on assaying all available DBS samples obtained for participants with a detectable HIV VL at one or more study visits, and discontinuing DBS assays for participants who were virologically suppressed at all study visits. Similar to a case (i.e. viraemic)–control (i.e. suppressed) study, extended within a longitudinal setting, this outcome-dependent sampling method ensured that data on the most informative patients (i.e. patients who were either viraemic at all visits or who switched VL status between visits) were included in the analysis, while maintaining statistical power by analysing all available samples from participants with at least one detectable HIV VL at any person-visit.^{8,21}

Differences in the proportion of quantifiable FTC-TP were compared in the viraemic and suppressed groups according to participant characteristics using Fisher's exact test or the χ^2 test, as appropriate. At baseline, χ^2 tests for trend were used to analyse the association between FTC-TP quantification and self-reported adherence. Generalized estimating equations, with a logit link, were utilized to estimate the OR of viral suppression comparing a quantifiable FTC-TP versus BLQ, while accounting for repeated measures (i.e. repeated HIV VL and DBS). The resulting OR was adjusted (aOR) for age, gender, race, BMI, estimated glomerular filtration rate (eGFR, calculated using the modification of diet in renal disease equation), CD4+ T cell count, ART class and duration of ART.⁸ An additional aOR including these variables plus tenofovir diphosphate was calculated to account for tenofovir exposure. The utility of self-reported adherence to predict a quantifiable FTC-TP in DBS was assessed by calculating the area under the receiver operating characteristic (ROC) curve using logistic regression for each of the three measures obtained (i.e. 3 days, 30 days and 3 months), and compared using the 3 day adherence measure as a reference. Statistical analyses were performed using SAS v9.4. A *P* value <0.05 was considered statistically significant.

Results

Patient population

The total study population enrolled was 807 participants, with 119 completing at least one visit (one pair of HIV VL and DBS samples), 244 completing at least two visits (two paired HIV VL and DBS samples) and 444 completing all three visits (three paired HIV VL and DBS samples). In total, 1936 DBS samples were collected in the entire cohort. Of these, 1199 DBS, obtained from 532 participants, were assayed, and 45 DBS samples obtained from 18 participants

who were not on an emtricitabine-containing regimen were excluded from the analysis, reducing our sample size to 514 participants for whom at least one DBS sample was assayed at any study visit.

Of the 514 participants in whom FTC-TP in DBS was quantified at any study visit, 503 had drug concentrations available at baseline. The demographic characteristics of these 503 participants who were on an emtricitabine-containing ART at the enrolment visit, according to viral suppression status, are shown in Table 1. Among these participants, 170 (34%) were viraemic and 333 (66%) were suppressed. Fifty-two (16%) out of the 333 participants who were suppressed at baseline became viraemic at one of the follow-up visits. In comparison, 88 (52%) out of the 170 participants who were viraemic at baseline became suppressed at one of the follow-up visits. Overall, the majority of participants were men,

with similar percentages of black, Hispanic and white participants between viraemic and suppressed participants. A larger percentage of viraemic participants were on an integrase strand-transfer inhibitor (INSTI)-based regimen when compared with suppressed participants (45% versus 32%), whereas a larger percentage of suppressed participants were on an NNRTI-based regimen in comparison with participants who were viraemic (35% versus 13%). Similarly, a larger percentage of viraemic participants were on a pharmacological booster such as ritonavir or cobicistat in comparison with participants who were suppressed (65% versus 44%). Longer duration of ART (i.e. >6 months) was more frequent in suppressed versus viraemic participants (88% versus 68%).

Table 1 shows the number of subjects reporting <100% adherence for each time period. Overall, a smaller percentage of suppressed individuals reported <100% adherence compared with

Table 1. Baseline characteristics of participants at the enrolment visit

Characteristic	Participants on FTC at baseline with available DBS (n = 503) ^a	
	viraemic (n = 170)	suppressed (n = 333)
Age	43 (33–51)	46 (38–52)
Sex		
male	153 (90)	278 (83)
female	17 (10)	55 (17)
Race		
black	34 (20)	63 (19)
Hispanic	34 (20)	61 (18)
other	8 (5)	16 (5)
white	94 (55)	193 (58)
BMI (kg/m ²)		
<18.5	7 (4)	14 (4)
18.5–25	78 (46)	132 (40)
>25–30	53 (31)	117 (35)
>30	32 (19)	68 (20)
ART class		
INSTI-based	77 (45)	106 (32)
multiclass	26 (15)	31 (9)
NNRTI-based	22 (13)	115 (35)
b/PI-based	45 (26)	81 (24)
Pharmacological booster		
no	60 (35)	187 (56)
yes	110 (65)	146 (44)
Time on ART (months)		
<1	17 (10)	4 (1)
1–3	22 (13)	22 (7)
>3–6	15 (9)	14 (4)
>6	116 (68)	293 (88)
Self-reported adherence <100% ^b		
3 days	36 (21)	36 (11)
30 days	82 (50)	132 (40)
3 months	98 (70)	178 (55)

Values are presented as n (%) or median (IQR).

FTC, emtricitabine; b/PI, boosted protease inhibitor.

^aFTC-TP concentrations in DBS were available in 503 participants at baseline from a total of 514 participants in whom FTC-TP was quantified at any study visit.

^bSelf-reported adherence in the preceding 30 days and 3 months was available for n = 495 and n = 464 participants, respectively.

Table 2. Proportion of quantifiable FTC-TP based on patient characteristics for participants at baseline

Variable	Viraemic (n = 170)			Suppressed (n = 333)		
	BLQ, n (%)	quantifiable FTC-TP, n (%)	P value ^a	BLQ, n (%)	quantifiable FTC-TP, n (%)	P value ^a
Sex						
male	34 (22)	119 (78)	0.236	6 (2)	272 (98)	0.623
female	6 (35)	11 (65)		2 (4)	53 (96)	
Race						
black	9 (26)	25 (74)	0.246	4 (6)	59 (94)	0.087
Hispanic	12 (35)	22 (65)		2 (3)	59 (97)	
other	1 (12)	7 (88)		0 (0)	16 (100)	
white	18 (19)	76 (81)		2 (1)	191 (99)	
BMI (kg/m ²)						
<18.5	1 (14)	6 (86)	0.796	0 (0)	14 (100)	0.81
18.5–25	21 (27)	57 (73)		4 (3)	128 (97)	
>25–30	12 (23)	41 (77)		2 (2)	115 (98)	
>30	6 (19)	26 (81)		2 (3)	66 (97)	
ART class						
INSTI based	15 (19)	62 (81)	0.092	3 (3)	103 (97)	0.474
multiclass	8 (31)	18 (69)		1 (3)	30 (97)	
NNRTI based	2 (9)	20 (91)		1 (1)	114 (99)	
b/PI based	15 (33)	30 (67)		3 (4)	78 (96)	
Pharmacological booster						
no	9 (15)	51 (85)	0.06	4 (2)	183 (98)	0.734
yes	31 (28)	79 (72)		4 (3)	142 (97)	
Time on ART (months)						
<1	3 (18)	14 (82)	0.173	1 (25)	3 (75)	0.157
1–3	2 (9)	20 (91)		0 (0)	22 (100)	
>3–6	2 (13)	13 (87)		0 (0)	14 (100)	
>6	33 (28)	83 (72)		7 (3)	266 (97)	

b/PI: boosted protease inhibitor.

^aEstimated using Fisher's exact test.

viraemic participants across all durations. Self-reported adherence was <100% at 3 days for 11% of the suppressed participants versus 21% of the viraemic participants.

FTC-TP and tenofovir diphosphate in DBS

Table 2 shows the quantification of FTC-TP in DBS for the study participants at the enrolment visit, categorized for whether they were viraemic or suppressed. The median (IQR) concentrations of FTC-TP among the viraemic and suppressed groups were 0.366 (0.278–0.459) and 0.367 pmol/punch (0.301–0.470), respectively. Suppressed patients had a higher percentage of quantifiable FTC-TP compared with viraemic patients ($P < 0.0001$). In an unadjusted analysis, there was no statistically significant difference in whether suppressed or viraemic participants had quantifiable FTC-TP versus BLQ based on gender, race, BMI, ART class, use of a pharmacological booster or duration of ART. Table 3 shows self-reported adherence results by FTC-TP quantification and by viral suppression status. In the viraemic group, the proportion of quantifiable FTC-TP in DBS increased with each adherence category for the 3 day, 30 day and 3 month adherence measures ($P < 0.0001$). In comparison, the proportion of quantifiable FTC-TP increased significantly

with each adherence category for only the 3 day adherence measure ($P = 0.004$) in the suppressed group.

Table 4 shows the quantification of FTC-TP according to the concentration of tenofovir diphosphate at baseline. Tenofovir diphosphate concentrations were <350 fmol/punch in 20 (4%), 350–699 fmol/punch in 32 (6%), 700–1249 fmol/punch in 103 (20%), 1250–1849 fmol/punch in 156 (31%) and ≥ 1850 fmol/punch in 192 (38%) of participants at baseline. Overall, the percentage of DBS where FTC-TP was BLQ decreased with every increasing category of tenofovir diphosphate.

FTC-TP in DBS as a predictor of viral suppression

A total of 1154 person-visits from 514 participants who had DBS obtained while on an emtricitabine-containing regimen at any time during the study were included in this analysis. Regarding association with viral suppression, the unadjusted OR (95% CI) of viral suppression when FTC-TP was quantifiable was 6.7 (4.3–10.5; $P < 0.0001$). After adjusting for age, gender, race, BMI, ART class, duration of ART, eGFR and CD4+ T cell count, the aOR for viral suppression when FTC-TP was quantifiable increased to 7.2 (4.3–12.0; $P < 0.0001$). After including tenofovir diphosphate in the

Table 3. Quantification of FTC-TP according to self-reported adherence and viraemia status at baseline

Percentage adherence	Viraemic (n = 170)			Suppressed (n = 333)		
	BLQ (n = 40)	quantifiable FTC-TP (n = 130)	P value	BLQ (n = 8)	quantifiable FTC-TP (n = 325)	P value
3 days (n = 503)						
<50	13	2		2	5	
50–84	4	5		0	9	
85–99	4	8		0	20	
100	19	115	<0.0001	6	291	0.004
30 days (n = 495) ^a						
<50	11	1		0	0	
50–84	9	14		1	12	
85–99	12	35		4	115	
100	8	73	<0.0001	3	197	0.115
3 months (n = 464) ^a						
<50	7	4		0	3	
50–84	16	17		3	27	
85–99	15	39		3	142	
100	1	42	<0.0001	2	143	0.052

Statistically significant *P* values are shown in bold.

^aSelf-reported adherence in the preceding 30 days and 3 months was available for *n* = 495 and *n* = 464 participants, respectively.

Table 4. Trend in FTC-TP quantification in DBS according to tenofovir diphosphate concentration at baseline

TFV-DP (fmol/punch)	FTC-TP		P value
	BLQ, <i>n</i> (%)	quantifiable, <i>n</i> (%)	
<350	17 (85)	3 (15)	—
350–699	11 (34)	21 (66)	—
700–1249	15 (15)	88 (85)	—
1250–1849	3 (2)	153 (98)	—
≥1850	2 (1)	190 (99)	<0.0001

TFV-DP, tenofovir diphosphate. Statistically significant *P* values are shown in bold.

multivariable analysis, the aOR of viral suppression when FTC-TP was quantifiable was 2.1 (1.2–4.0; *P* < 0.015).

Self-reported adherence as predictor of FTC-TP quantification

The sensitivity and specificity of self-reported adherence as a predictor of FTC-TP quantification in DBS is shown in Figure 1. The area under the ROC curve for 3 day self-reported adherence was 0.82 (95% CI 0.75–0.88, *P* = 0.011), which compares to 3 month self-reported adherence at 0.70 (95% CI 0.62–0.77, *P* = 0.004) and 30 day self-reported adherence at 0.79 (95% CI 0.71–0.86; *P* = 0.32), though the latter did not reach statistical significance.

Discussion

In this study, we demonstrated that the quantification of FTC-TP in DBS is predictive of virological suppression in PLWH and is an

effective biomarker to determine recent adherence to ART among patients on an emtricitabine-containing regimen. To our knowledge, this is the first documentation of the use of FTC-TP in DBS in a cohort of participants living with HIV, as prior studies involved only HIV-negative volunteers. The multivariable adjusted analysis showed that quantifiable FTC-TP was significantly associated with virological suppression, with an adjusted odds of suppression >7-fold higher in those with quantifiable FTC-TP compared with those with BLQ FTC-TP. This remained significant after adjusting for tenofovir diphosphate [aOR 2.1 (95% CI 1.2–4.0); *P* < 0.015]. Therefore, short-term adherence monitoring for FTC-TP is critical and provides additional information on adherence patterns, even after accounting for cumulative adherence with tenofovir diphosphate. Combined, these two markers could be utilized to obtain a complete picture of adherence to optimize adherence counselling and virological suppression among PLWH.

In the unadjusted analysis, there was no statistically significant difference in baseline characteristics according to whether participants had quantifiable FTC-TP versus BLQ for either the suppressed or the viraemic groups. In both, the proportion of participants with FTC-TP in DBS increased with each adherence category in the 3 day, 30 day and 3 month adherence measures. For viraemic participants, there was a statistically significant difference noted in all three adherence categories, but only 3 day self-reported adherence remained significant for those who were suppressed. This suggests that small changes in variability in adherence over 30 days and 3 months may be harder to detect using a short-term adherence marker in a virologically suppressed individual. Similar findings were observed when the ROC curves for self-reported adherence and FTC-TP were evaluated, in which the largest estimated ROC for quantifiable FTC-TP was observed with 3 day self-reported adherence. Collectively, these findings demonstrate that FTC-TP is a better measure of short-term adherence, as previously observed in individuals without HIV infection.¹¹

Figure 1. Baseline ROC curves for self-reported adherence as a predictor of FTC-TP quantification in DBS. Lines represent the area under the ROC curves (AUC) for 3 day (0.82), 30 day (0.79) and 3 month (0.70) self-reported adherence. SR, self-reported.

Prior studies in HIV-negative volunteers have verified the association between the plasma half-life of emtricitabine and quantification of FTC-TP in DBS.¹⁴ The Cell-PrEP study found similar half-lives of FTC-TP in DBS between PLWH and HIV-negative volunteers, with almost all at BLQ at washout 5 days after the last dose.¹¹ Similar findings were observed in the DOT-DBS study, in which 100% FTC-TP detection was found in participants taking daily doses.¹⁸ The iPrEx OLE and ATN 110 studies demonstrated a strong association between tenofovir diphosphate and FTC-TP in 746 paired plasma and DBS samples.¹¹ These results were similar to those found in our study, showing a strong association between FTC-TP quantification and higher concentrations of tenofovir diphosphate. However, in our study, 85% of participants had quantifiable FTC-TP at tenofovir diphosphate concentrations >700 fmol/punch (the expected concentration for four or more doses per week in people without HIV), which was lower than the 96% of HIV-negative volunteers with quantifiable FTC-TP at the same tenofovir diphosphate concentrations.¹¹ This likely reflects the higher variability in adherence patterns among PLWH in clinical practice, as opposed to the expected higher levels of adherence among participants in a clinical trial setting. However, other factors inherent to HIV infection, such as drug-drug interactions, pharmacokinetic differences and/or residual inflammation, could have also influenced these findings and require further study.

The major clinical implication of this study is that FTC-TP measured in DBS can be utilized as an objective measurement

of short-term adherence to ART among PLWH. Given that the majority of current first-line regimens include emtricitabine as a major component, typically combined with tenofovir disoproxil fumarate or tenofovir alafenamide,^{22,23} FTC-TP in DBS has the potential to complement tenofovir diphosphate as a highly useful biomarker of ART adherence in clinical practice. We found that 3 day self-reported adherence to emtricitabine best predicted the quantification of FTC-TP in DBS, which likely reflects the short half-life of FTC-TP at 35 h.¹¹ Thus, this can distinguish a patient's recent adherence patterns prior to a clinical visit.²⁴ Our results also showed a strong association between FTC-TP quantification and higher tenofovir diphosphate concentrations. A cumulative adherence assessment is critical to exclude transient improved adherence prior to a clinic visit, termed 'white coat' adherence,²⁴ and could overcome the limitations of self-reported adherence or pill counts to detect recent episodes of suboptimal adherence and prevent virological failure. For example, we observed quantifiable FTC-TP in 15% (3/20) of subjects with low tenofovir diphosphate (<350 fmol/punch), suggesting 'white coat' dosing.

The strengths of this study include the prospective analysis of a large clinical cohort in a clinical setting. In addition, FTC-TP was associated with self-reported adherence in various time frames, allowing analysis of the utility of this biomarker over different time periods. Limitations include the possible overestimation of self-reported adherence,³ which was particularly evident in the larger proportion of participants who reported 100% 3 day adherence and had FTC-TP concentrations BLQ, a discrepancy which could have skewed our results. Despite this, self-report continues to be widely used in routine clinical practice and thus our focus on comparing FTC-TP with this adherence assessment. In addition, although the outcome-dependent sampling approach could have influenced the strength of our association, the focus on viral suppression as a reasonably rare outcome allowed us to longitudinally extend our case-control design, while maintaining statistical power.

In summary, we determined that FTC-TP in DBS is associated with virological suppression in PLWH and that it is best predicted by 3 day self-reported adherence. Future studies that can prospectively evaluate this biomarker (alone and in conjunction with tenofovir diphosphate) to identify early changes in adherence in clinical practice are required.

Acknowledgements

We would like to thank the study participants, the director of the HIV programme (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. We would also like to thank the personnel at the Colorado Antiviral Pharmacology Laboratory for their invaluable assistance and support of this study.

Funding

This work was supported by the National Institutes of Health (K23 AI104315 to J. C.-M., R21 AI124859 to J. C.-M. and R01 AI122298 to P. L. A.). P. L. A. receives support (paid to their institution) for investigator-initiated studies from Gilead Sciences.

Transparency declarations

None to declare.

References

- 1 Cohen MS, Chen YQ, McCauley M *et al*. Antiretroviral therapy for the prevention of HIV-1 transmission. *N Engl J Med* 2016; **375**: 830–9.
- 2 Castillo-Mancilla JR, Haberer JE. Adherence measurements in HIV: new advancements in pharmacologic methods and real-time monitoring. *Curr HIV/AIDS Rep* 2018; **15**: 49–59.
- 3 Simoni JM, Kurth AE, Pearson CR *et al*. Self-report measures of antiretroviral therapy adherence: a review with recommendations for HIV research and clinical management. *AIDS Behav* 2006; **10**: 227–45.
- 4 Okatch H, Beiter K, Eby J *et al*. Brief report: apparent antiretroviral overadherence by pill count is associated with HIV treatment failure in adolescents. *J Acquir Immune Defic Syndr* 2016; **72**: 542–5.
- 5 Castillo-Mancilla JR, Zheng JH, Rower JE *et al*. Tenofovir, emtricitabine, and tenofovir diphosphate in dried blood spots for determining recent and cumulative drug exposure. *AIDS Res Hum Retroviruses* 2013; **29**: 384–90.
- 6 Grant RM, Anderson PL, McMahan V *et al*. Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. *Lancet Infect Dis* 2014; **14**: 820–9.
- 7 Liu AY, Cohen SE, Vittinghoff E *et al*. Preexposure prophylaxis for HIV infection integrated with municipal- and community-based sexual health services. *JAMA Intern Med* 2016; **176**: 75–84.
- 8 Castillo-Mancilla J, Morrow M, Coyle RP *et al*. Tenofovir diphosphate in dried blood spots is strongly associated with viral suppression in individuals with HIV infection. *Clin Infect Dis* 2018; doi:10.1093/cid/ciy708.
- 9 Castillo-Mancilla JR, Searls K, Caraway P *et al*. Short communication: tenofovir diphosphate in dried blood spots as an objective measure of adherence in HIV-infected women. *AIDS Res Hum Retroviruses* 2015; **31**: 428–32.
- 10 Warne P, Robbins R, Anderson P *et al*. Utility of dried blood spot-derived ARV biomarkers as an objective measure of treatment adherence in South Africa. In: *IAPAC 10th International Conference on HIV Treatment and Prevention Adherence 2015, Miami, FL, USA*. Abstract 241, 2015.
- 11 Castillo-Mancilla J, Seifert S, Campbell K *et al*. Emtricitabine-triphosphate in dried blood spots as a marker of recent dosing. *Antimicrob Agents Chemother* 2016; **60**: 6692–7.
- 12 Zheng JH, Rower C, McAllister K *et al*. Application of an intracellular assay for determination of tenofovir-diphosphate and emtricitabine-triphosphate from erythrocytes using dried blood spots. *J Pharm Biomed Anal* 2016; **122**: 16–20.
- 13 Amico KR, Fisher WA, Cornman DH *et al*. Visual analog scale of ART adherence: association with 3-day self-report and adherence barriers. *J Acquir Immune Defic Syndr* 2006; **42**: 455–9.
- 14 Giordano TP, Guzman D, Clark R *et al*. Measuring adherence to antiretroviral therapy in a diverse population using a visual analogue scale. *HIV Clin Trials* 2004; **5**: 74–9.
- 15 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**: e01710–7.
- 16 Viswanathan S, Justice AC, Alexander GC *et al*. Adherence and HIV RNA suppression in the current era of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2015; **69**: 493–8.
- 17 Koblin AB, Sheth NU. Levels of adherence required for virologic suppression among newer antiretroviral medications. *Ann Pharmacother* 2011; **45**: 372–9.
- 18 Bangsberg DR, Kroetz DL, Deeks SG. Adherence-resistance relationships to combination HIV antiretroviral therapy. *Curr HIV/AIDS Rep* 2007; **4**: 65–72.
- 19 Gardner EM, Burman WJ, Steiner JF *et al*. Antiretroviral medication adherence and the development of class-specific antiretroviral resistance. *AIDS* 2009; **23**: 1035–46.
- 20 Viswanathan S, Detels R, Mehta SH *et al*. Level of adherence and HIV RNA suppression in the current era of highly active antiretroviral therapy (HAART). *AIDS Behav* 2015; **19**: 601–11.
- 21 Schildcrout JS, Schisterman EF, Mercaldo ND *et al*. Extending the case-control design to longitudinal data: stratified sampling based on repeated binary outcomes. *Epidemiology* 2018; **29**: 67–75.
- 22 Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV*. Department of Health and Human Services, 2018. <http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf>.
- 23 Saag MS, Benson CA, Gandhi RT *et al*. Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2018 recommendations of the international antiviral society–USA panel. *JAMA* 2018; **320**: 379–96.
- 24 Podsadecki TJ, Vrijens BC, Tousset EP *et al*. “White coat compliance” limits the reliability of therapeutic drug monitoring in HIV-1-infected patients. *HIV Clin Trials* 2008; **9**: 238–46.