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Higher tenofovir exposure is associated with longitudinal declines in kidney function in women living with HIV

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Abstract

Objective—Tenofovir disoproxil fumarate is a commonly used antiretroviral drug, but risk factors for tenofovir (TFV)-associated kidney disease are not fully understood. We used intensive pharmacokinetic studies in a cohort of HIV-infected women on TFV-based therapy to study the relationship between TFV exposure and subsequent kidney function.

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Design—This is a nested study within the Women's Interagency HIV Study, a multicenter, prospective cohort of HIV-infected women. Participants on TFV-based therapy underwent 24-h intensive pharmacokinetic sampling after witnessed dose. Kidney function was measured over the succeeding 7 years by serum creatinine [estimated glomerular filtration rate calculated by serum creatinine (eGFRcr)].

Methods—Multivariable linear mixed models evaluated the relationship of baseline TFV area under the-time concentration curves (AUCs) with subsequent changes in kidney function. Covariates included age, diabetes, hypertension, race, BMI, ritonavir use, duration of TFV exposure, current $CD4^+$ cell count, and HIV viral load.

Results—Of the 105 participants, persons within the highest baseline TFV AUC tertile had significantly lower eGFRcr compared with those in the lowest tertile (mean±stan-standard error: 80 ± 4.3 vs. 104 ± 2.5 ml/min per 1.73 m^2, $P<0.0001$). By year 7, this difference widened (72 ± 4.9 vs. 105 ± 2.9, $P<0.0001$). After multivariable adjustment, TFV AUC in the highest tertile remained associated with lower eGFRcr relative to values in the lowest tertile at both baseline (-15 ml/min per 1.73 m^2, $P=0.0047$) and year 7 (-23 ml/min per 1.73 m^2, $P=0.0002$).

Conclusion—Through intensive TFV pharmacokinetic sampling, we found a strong association between greater TFV exposure and subsequent decline in kidney function. Variations in TFV drug exposure may partially account for subsequent nephrotoxicity in persons infected with HIV.

Introduction

Tenofovir disoproxil fumarate (TDF) is one of the most commonly used antiretroviral drugs for the treatment and prevention of HIV infection. Currently, TDF is a component in several one-pill once-a-day combination antiretroviral therapy (ART) formulations and is recommended for first line ART regimens by international guidelines [1]. As TDF formulations become increasingly available in global settings where the burden of HIV is highest, and as the HIV-infected population ages, it is vital to gain a better understanding of the factors that contribute to TDF toxicity.

Systemic exposure to drug is optimally characterized by pharmacokinetic methods, but prior pharmacokinetic studies of TDF have been limited to populations that differ in race, sex, and comorbidity from large populations of HIV patients [2]. Additionally, these prior pharmacokinetic studies have not examined the relationship between variations in TDF exposure and subsequent kidney function. Determination of kidney function following intensive pharmacokinetic analyses is an important means to determine whether TDF exposure is predictive of subsequent kidney impairment.

Although TDF is highly effective and well tolerated in most recipients, its use has been associated with reduced kidney function and proteinuria [3–8], adverse consequences of treatment that can lead to discontinuation of the drug, and which may not be reversible after

treatment discontinuation [4,7,9]. Although formulated as TDF to promote oral absorption and tissue delivery, pharmacokinetic studies often measure tenofovir (TFV), the active form of the drug in plasma, or its' phosphorylated metabolites. For many drugs, the occurrence of adverse effects is directly related to systemic exposure to the drug [defined as areas under the time concentration curve (AUC)]. TFV, like the majority of drugs, demonstrates significant interindividual variability in plasma drug and trough levels that can be affected by a variety of clinical factors [10–14]. Understanding the pharmacokinetics of TFV is important for providing insight into whether the degree of drug exposure is associated with toxicity. Interindividual variability in TFV exposure has been hypothesized to be a determinate of kidney injury, but studies to date examining this relationship have been limited by small sample sizes and short duration of observations, by study populations with limited generalizability, and use of surrogate assessments of TFV pharmacokinetics [15,16].

The primary objective of this study was to determine if TFV exposure, as measured by TFV AUCs is associated with kidney function over a long period of observation. Our hypothesis was that TFV exposure would be independently associated with a decline in kidney function over time.

Methods

Study design and population

The Women's Interagency HIV Study (WIHS) is a large, multicenter, prospective cohort study of HIV-infected women and at-risk HIV-uninfected women in the United States [17], ongoing since 1993. The WIHS is representative of US women living with HIV in terms of demographic and clinical parameters. We previously described the 'WIHS Intensive PK Study' [18,19], which enrolled 480 HIV-infected women on varying ART regimens. This intensive pharmacokinetic assessment, conducted from 2004 to 2008, included 12–24-h sampling of antiretroviral plasma levels after administration of a dose witnessed by study team members. Participant data were included in this analysis for women who reported steady-state use of TDF (i.e. TDF use for at least 6 months prior to pharmacokinetic evaluation), underwent 24-h intensive pharmacokinetic sampling for plasma TFV levels, and had at least one estimated glomerular filtration rate calculated by serum creatinine (eGFRcr) from a WIHS visit after the pharmacokinetic study. Participants were not excluded for ongoing recreational drug use. Laboratory studies, physical examinations, demographic information, self-reported ARV adherence, and other characteristics were obtained every 6 months on WIHS participants as part of participation in the overall prospective cohort study. Institutional review boards at all participating institutions approved the study and informed consent materials. Written informed consent for the pharmacokinetic study was obtained from each study participant. Participants were continued to be followed (and included in this study) even if they discontinued TDF, and they were only censored at loss-to-follow-up.

Intensive pharmacokinetic protocol methods

Pharmacokinetic protocols were conducted in clinical research centers or other facilities associated with collaborating WIHS sites. The TFV measurement procedure has been previously described [12], but important details are included here. Participant's typical diet

was determined via phone interview 2–3 days prior to the pharmacokinetic study. That diet was then provided during the pharmacokinetic protocol. Patients used their routine medications during the protocol, and these were recorded by study staff. Plasma samples were drawn at timed intervals over 24 h for drug levels under conditions of actual use, including providing the participants' home medications, and processed within 30 min of sample collection. All participants received their standard dose of TDF and TFV levels were measured in plasma specimens collected at 0, 4, 8, 15, 18, and 24 h after witnessed dose. Participants were seen for the pharmacokinetic visit within 6 weeks of a core WIHS cohort visit, thus temporally linking measures between the WIHS data and pharmacokinetic data. Weight and medication use were collected at both the intensive pharmacokinetic and core WIHS visit, whereas data on comorbidities, HIV RNA level, CD4^{+} cell counts, and serum creatinine were collected during the core WIHS visits.

Laboratory procedures

Plasma levels of TFV were determined by liquid chromatography/tandem mass spectrometry with TDF-d6 as the internal standard [20]. The plasma sample was pretreated with trifluoroacetic acid for protein precipitation before injecting into the Micromass Quattro Ultima (Waters Corporation, Milford Massachusetts, USA) liquid chromatography/ tandem mass spectrometry system as previously described [12,20]. The assay has been validated from 10 to 1000 ng/ml of TFV with a coefficient of variation less than 15% for quality control samples at low, medium, and high concentrations.

Covariates used for adjustment

As in our prior analysis [12], the following baseline covariates were included in all multivariable models: age, race/ethnicity, BMI, diagnosis of diabetes or hypertension, self-reported prior duration of TDF usage, concurrent ritonavir use, current CD4^{+} cell count (log transformed), and concurrent HIV viral load (detectable vs. nondetectable, defined as above or below assay thresholds of 120, 80 or 50 copies/mL, depending on assay used by WIHS at the time of the relevant visit).

Primary predictor

AUCs were used to estimate TDF exposure over the 24-h dosing interval (once at baseline); these were calculated for each individual using the trapezoidal rule [21]. For calculation of the AUC, any observations with TFV concentrations below the lower limit of quantification (10 ng/ml) were replaced by 0 ng/ml; 10 individuals had undetectable TFV at baseline at the beginning of the intensive pharmacokinetic study. One individual had a single value that was undetectable at a time other than baseline.

Outcome

The Chronic Kidney Disease Epidemiology Collaboration equation using serum creatinine was used to estimate glomerular filtration rate (eGFRcr in ml/min per 1.73 m^2) [12,22]. We analyzed changes in eGFRcr as a continuous outcome, expressed as annual differences in ml/min per 1.73 m^2/year over approximately 7 years of WIHS follow-up.

Statistical analysis

We stratified participants into three categories based on tertile of TFV AUC, and compared demographic and baseline clinical characteristics using the χ^2 and Kruskal–Wallis tests for categorical and continuous variables, respectively. Kernel density estimates were used to construct smoothed density curves to examine distributions of baseline eGFRcr levels, and were compared by tertile of TFV AUC using Levene's test for homogeneity of variance. Multivariable linear mixed effect models were used to evaluate the relationship of baseline TFV AUC with subsequent change in kidney function, with random intercepts and slopes using an unstructured variance–covariance matrix for modeling. Graphical examination of the trajectories found curvilinear changes in eGFRcr. Therefore, we modeled eGFRcr using linear splines, with potentially different slopes during the earlier and later follow-up intervals (0–3.5 and 3.5–7 years). We analyzed tertiles of TFV AUC with tertile 1 (lowest AUC) as the reference category for all analyses. We also evaluated levels of TFV AUC (above vs. below median) and baseline eGFRcr (<90 vs. >90 ml/min/1.73 m^2) jointly, to determine whether the association of TFV AUC with subsequent change in kidney function varied by level of baseline eGFRcr.

We used relative risk regression with a modified Poisson approach to examine associations of TFV AUC with incident chronic kidney disease (CKD) [23]. We analyzed TFV AUC both continuously (per 10% increase) and categorically (using tertiles). We defined incident CKD using eGFR by creatinine, calculated using the Chronic Kidney Disease Epidemiology Collaboration equation as eGFR less than 50, 60, or 70 at any two consecutive visits over approximately 7 years after baseline. All analyses were conducted using SAS (version 9.4, Cary, North Carolina, USA).

Results

The baseline demographic and clinical characteristics of participants in the study, stratified by TFV AUC tertile, are summarized in Table 1. A total of 117 women underwent intensive pharmacokinetic sampling for TFV, but 12 women were excluded from analysis because of missing data (including no eGFRcr) or lack of subsequent measures. The recorded dose for TDF was 300 mg once daily in all of the participants' ART regimens. Among the 105 women who contributed data to this analysis, the mean age was 43 years (range 22–65), 68% were African-American, 13% white, and 20% of other race; 25 (24%) self-identified as Hispanic ethnicity. Women with TFV AUC in the highest tertile were older and had lower median BMI relative to those with TFV AUC in the lower tertiles. Concurrent CD4$^+$ cell counts were lower and detectable HIV RNA was more prevalent among women with TFV AUC in the highest tertile than those with lower AUCs, but these differences were not statistically significant.

We examined the distribution of eGFRcr at baseline, stratified by TFV AUC tertile (tertile 1 has the lowest AUC, tertile 2 had intermediate AUC values, and tertile 3 had the highest TFV AUC values) (Fig. 1). The median eGFRcr at baseline was 104 ml/min per 1.73 m^2 in tertile 1, 97 ml/min per 1.73 m^2 in tertile 2, and 78 ml/min per 1.73 m^2 in tertile 3 of TFV AUC. The spread of eGFRcr levels was considerably wider among participants with

exposures in tertile 3 compared with those in tertiles 1 and 2 (P=0.0021 by test for homogeneity of variance).

We next examined trajectories of change in eGFRcr over time by TFV AUC tertile (Fig. 2). Mean eGFRcr was lowest among those with highest TFV AUC at baseline (mean ml/min/ 1.73 m^2±standard error: 80±4.3 vs. 104±2.5 for highest vs. lowest tertile, P<0.0001), and these differences widened over time (year 7: 72±4.9 vs. 105±2.9, P<0.0001). The trajectories of eGFRcr among women in tertiles 1 and 2 showed similar curvilinear changes, with increases in eGFRcr apparent during years 0–3.5, followed by decreases in years 3.5–7. By contrast, women with TFV AUC in tertile 3 showed decreases in eGFRcr throughout the entire follow-up period. Analyses confirmed that tertile 3 (during years 0–3.5) had significantly larger declines in eGFRcr than tertile 1 (−1.87 ml/min per 1.73 m^2/year, P=0.029; Table 2), whereas there was no statistically significant difference between tertiles 1 and 2. This more pronounced decline in eGFRcr for women whose TFV AUC were in tertile 3 remained statistically significant after multivariable adjustment for all cofactors. During the later period (years 3.5–7) significant decreases in eGFRcr occurred for women with TFV AUC values in all three tertiles, ranging from 1.4 to 2.4 ml/min per 1.73 m^2/year (all P<0.01), and no statistically significant differences were detected across tertiles. Supplemental Table 1, http://links.lww.com/QAD/A829 shows eGFRcr estimates from multivariate linear mixed models using piecewise linear spline, separated yearly.

We then restricted the analysis to include only those participants who had detectable TFV at the start of the study (N=95), which resulted in nine individuals being removed from tertile 1 and one individual being removed from tertile 3. Results were similar after excluding those with undetectable TFV, in that tertile 1 showed a 2.1 ml/min per 1.73 m^2 [95% confidence interval (CI) 0.92–3.4 ml/min per 1.73 m^2] average annual increase in eGFR over years 0– 3.5, and a 1.5 ml/min per 1.73 m^2 (95% CI −2.6 to −0.53 ml/min per 1.73 m^2) average annual decline in eGFR over years 3.5–7. In multivariable adjusted analysis, over years 0– 3.5, both tertiles 2 and 3 had smaller gains on average relative to tertile 1. Being in tertile 2 was associated with a 2.3 ml/min per 1.73 m^2 difference (95% CI −4.2 to −0.45 ml/min per 1.73 m^2) and being in tertile 3 was associated with a 3.2 ml/min/1.73 m^2 difference (95% CI −5.0 to −1.38 ml/min per 1.73 m^2), relative to tertile 1. Over years 3.5–7, differences in annual change narrowed and were no longer statistically different between tertiles. We also evaluated the association of TFV AUC with incident CKD using various eGFRcr (50, 60, or 70 ml/min/1.73 m^2) thresholds (Supplemental Table 2, http://links.lww.com/QAD/A829). In unadjusted analysis, each 10% increase in the TFV AUC was associated with a 37% increased risk of eGFR less than 50 ml/min per 1.73 m^2, a 30% increased risk of eGFR less than 60 ml/min per 1.73 m^2, and an 18% increased risk of eGFR less than 70 ml/min per 1.73 m^2 (all P<.05). Although we had reduced power because of the small numbers of incident cases, we found that greater TFV AUC remained associated with a 22% increased risk of eGFR less than 70 ml/min per 1.73 m^2 after multivariable adjustment. When defined as eGFR less than 78 ml/min per 1.73 m^2, rates of CKD ranged from 5.7% in tertile 1 to 29% in tertile 3, resulting in a five-fold increased risk of CKD for those in tertile 3 even after multivariable adjustment. Finally, we examined the effect of stratifying by both TFV AUC and baseline eGFRcr, comparing trajectories of eGFRcr change over time (Fig. 3). We

first considered persons with eGFRcr less than 90 ml/min per 1.73 m^2 at baseline (Fig. 3a); the eGFRcr trajectory appeared parallel in the lower (*n*=15) and higher (*n*=31) TFV AUC groups although the absolute level of eGFRcr was consistently lower in the higher TFV AUC group. In persons with eGFRcr more than 90 ml/min per 1.73 m^2 at baseline (Fig. 3b), the initial eGFRcr was similar in those with lower (*n*=37) and higher (*n*=22) TFV AUC, but those with higher TFV AUC experienced a greater decline in eGFRcr (*P*=0.025 for years 0–3.5 and *P*=0.71 for years 3.5–7). Supplemental Table 3, http://links.lww.com/QAD/A829 shows eGFRcr estimates from multivariate linear mixed models using piecewise linear spline, separated yearly and stratified by both baseline eGFRcr and TFV AUC.

Discussion

This is the first report to demonstrate that greater TFV exposure, measured by intensive 24-h pharmacokinetic assessment, is strongly associated with more rapid declines in kidney function over time. We observed a significant decline in kidney function over the first 3.5 years of follow-up, which did not normalize by year 7. Higher TFV exposure remained independently associated with more impaired kidney function longitudinally, even after controlling for duration of prior TFV exposure, baseline kidney function, and other relevant risk factors that may influence kidney function. The association between eGFRcr estimated at the time of intensive pharmacokinetic and TFV exposure as represented by AUCs in this study could be attributable to the effect of reduced kidney function on TFV clearance or the effect of higher TFV concentrations on kidney function. However, the longitudinal association of higher TFV AUC with a more rapid subsequent decline in kidney function provides strong evidence that greater exposure to TFV can injure the kidney over time.

These data indicate that the extent of TFV exposure may influence the occurrence of kidney injury, which may have implications for dosing and prescribing strategies. Our group and others have identified clinical factors that increase TFV AUC in intensive pharmacokinetic studies, including concomitant ritonavir use, pre-TDF impairment of eGFRcr, lower body weight, and increasing age [12]. Some have advocated for modifications in TDF dosing, particularly with the use of specific protease inhibitors [24]; given the results of our study, such alternative dosing regimens should be further explored to understand whether they may alleviate subsequent kidney toxicity for some individuals on this commonly used antiretroviral agent. We need a better understanding of the factors that determine the pharmacokinetics and pharmacodynamics of TFV and the extent to which these are modified by changes in dosing. Modification to dosing must be evaluated while also ensuring that control of HIV replication is maintained. Our study has shown that there is tremendous between-person variability in exposure to TFV, despite fixed dose regimens, a fact that is important in understanding TDF's potential toxicity. Determining TFV AUC in the clinical setting may be impractical, but identifying other strategies to predict toxicity should be explored in future studies. One possibility is the use of single trough levels that have been correlated with total daily TFV exposure.

In persons at risk for progression of kidney disease in the setting of TDF use, other agents can be considered as part of the backbone ART regimen in lieu of TDF. For example, abacavir may lead to greater improvements in albuminuria and proteinuria in persons taking

ART compared with those on TDF-based regimens [25]. Other therapeutic approaches may lead to lower rates of kidney injury because of lower systemic exposure to the TDF moiety. One such opportunity could be tenofovir alafenamide fumarate (TAF), a novel prodrug of TFV that has been reported as having the potential benefits of lower kidney and bone toxicity without a compromise in treatment efficacy [26–30]. Pharmacokinetic studies demonstrated that a TAF dose of 25 mg (compared with 300 mg of TDF) resulted in an approximate 86% reduction in systemic exposure and a seven-fold increase in the mean concentration of intracellular peripheral blood mononuclear cell TFV diphosphate [31]. In addition, TFV from TDF is renally secreted via organic anion transporters (OAT1 and OAT3), whereas TAF has not been shown to significantly interact with OAT or result in OAT-dependent cytotoxicity *in vitro* [29]. However, it is unknown whether TAF treatment will lead to lower kidney tubular cell levels of TFV than TDF since TFV is a substrate for OAT. This idea has been suggested given the lower systemic exposure to TFV seen with TAF and clinical trials demonstrating lower levels of kidney injury markers for patients using TAF relative to TDF [26–28,31]. If it is felt that the mechanism of kidney tubular cell injury is attributable to mitochondrial injury, as supported by in-vitro studies [32–36], then intracellular concentrations of drug would then seem to be highly relevant. Longitudinal data in real-world settings will be required to determine whether TAF will be a reasonable therapeutic option for persons on TDF who develop kidney or bone toxicity.

There were several strengths to this study. First, this is the largest study to perform intensive pharmacokinetic measurements in patients on TDF-based regimens. We were able to follow these participants for an average of more than 7 years after completion of exposure determination, allowing a far more comprehensive assessment of the potential kidney complications of higher levels of TFV exposure. These study results were obtained from an ethnically and clinically diverse cohort comprised mostly of racial/ethnic minority women – a group that has been traditionally underrepresented in HIV research [37–39].

There were also some limitations to the interpretation of these data. An important assumption underlying our analysis was that TFV AUC does not change over time. This may not be true, and in the setting of declining eGFRcr, it is possible that TFV AUC may be gradually increasing; this could contribute to further reduction of kidney function in a vicious self-perpetuating cycle. In fact, our data support this, given the cross-sectional associations of lower eGFRcr with higher TFV exposure at baseline. It is, therefore, possible that our calculations of the relationship between eGFRcr and TFV AUC are underestimated. Also, we only measured glomerular function, and not tubular function. TFV-associated kidney disease may be a result of tubular injury [40,41] but the impact on eGFRcr over longer periods of exposure is not clear. This specific cohort does not yet have serial measurements of biomarkers of tubular injury such as IL-18, kidney injury molecule-1, or α-1 microglobulin. More specific and early markers of kidney toxicity such as these would enable better determination of the exact mechanisms underlying TFV-induced injury. We were not able to control for genetic factors that may play a role in determining TFV exposure and may be either confounding or modifying the relationship between TFV AUC and subsequent eGFRcr. A number of polymorphisms have been identified that result in increased intracellular TFV concentration or increased proximal tubular injury [42,43], but

whether these factors or different ones are relevant to the diverse population in this study is not well characterized. The study was limited to women, and therefore the results may pertain to women only. Nearly all the women in this study received coadministration of emtricitabine as part of their antiretroviral regimens and an impact of emtricitabine on the outcome cannot be definitely ruled out. Notably, a higher proportion of individuals in tertile 3 had less than 7 years of follow-up in the study; if they dropped out of the cohort because they were sicker or had worse kidney function, then our estimates for the decline in eGFRcr would be underestimated in this group. However, when we evaluated the association of TFV AUC with incident CKD using various eGFRcr (50, 60, or 70 ml/min per 1.73 m^2) binary thresholds (Supplemental Table 2, http://links.lww.com/QAD/A829), higher TFV AUC remained associated with an increased risk of CKD.

In summary, we present compelling evidence suggesting that higher levels of exposure to TFV may partially account for subsequent declines in kidney function over time in a cohort of diverse women living with HIV. There is evidence for more rapid progression of kidney function decline in individuals with higher TFV exposure. There is also likely an effect of reduced kidney function leading to higher degrees of TFV exposure, which may operate in a cyclic feed-forward mechanism. These data support further studies exploring the factors that determine TFV pharmacokinetics as well as investigations regarding dosing reduction and novel treatment strategies to optimize drug exposure while minimizing drug toxicity in select high-risk populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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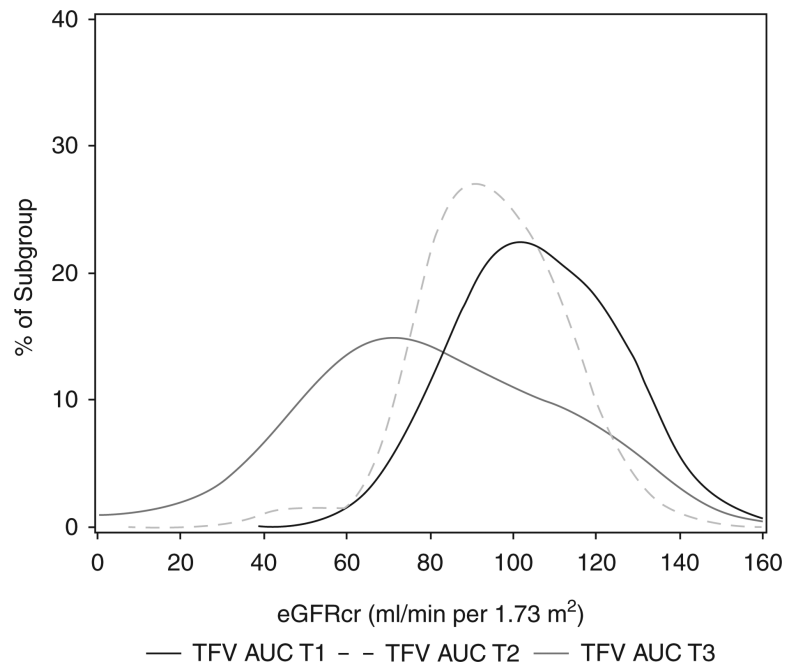
**Fig. 1.**
Distribution of baseline estimated glomerular filtration rate calculated by serum creatinine (ml/min per 1.73 m^2) in HIV-infected women, stratified by baseline tenofovir area-under-the-time-concentration-curve tertile.
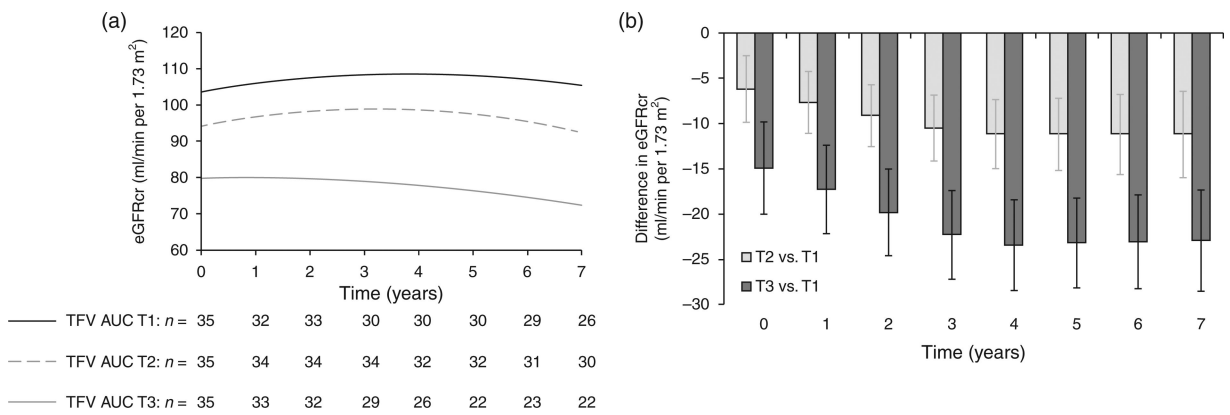
Fig. 2.
(a) Trajectory of eGFRcr (ml/min per 1.73 m^2) in HIV-infected women over time (unadjusted linear mixed model), showing mean. (b) This panel shows adjusted trajectory of eGFRcr by tertile of TFV AUC with estimates from a linear mixed model with piecewise linear spline, where T1, TFV AUC tertile 1; T2, TFV AUC tertile 2, and T3, TFV AUC tertile 3. AUC, area-under-the-time-concentration-curve; eGFRcr, estimated glomerular filtration rate calculated by serum creatinine; TFV, tenofovir.

(a)

(b)
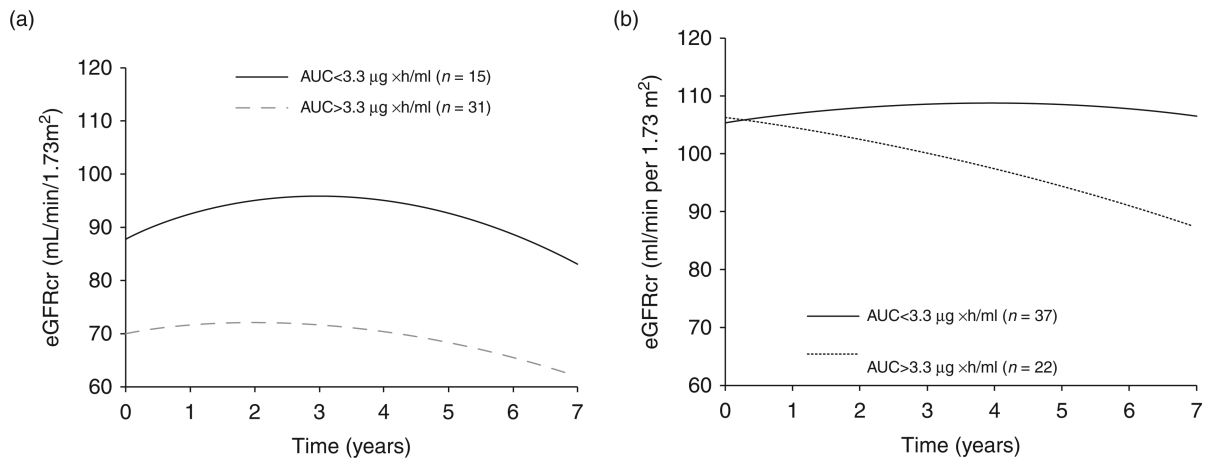
Fig. 3.
(a) Trajectory of eGFRcr (ml/min per 1.73 m^2) over time in patients with baseline GFRcr less than 90 ml/min per 1.73 m^2. (b) Baseline eGFRcr more than 90 ml/min per 1.73 m^2 stratified into groups of low TFV AUC (<3.3 μg × h/ml) vs. high TFV AUC (>3.3 μg × h/ml). AUC, area-under-the-time-concentration-curve; eGFRcr, estimated glomerular filtration rate calculated by serum creatinine; TFV, tenofovir.

Table 1

Baseline characteristics of study participants by tertile of tenofovir disoproxil fumarate area under the time concentration curve ($n = 105$ total).

Parameter	TFV AUC tertile 1 ($n = 35$)	TFV AUC tertile 2 ($n = 35$)	TFV AUC tertile 3 ($n = 35$)	P value
TFV AUC range (ng*h/ml)	1031–2640	2646–3922	4009–13 911	
Age (years)	41 (31, 47)	42 (34, 49)	47 (44, 51)	0.0004
Race				0.32
African-American	22 (63%)	27 (77%)	22 (63%)	
White	7 (20%)	3 (9%)	3 (9%)	
Other	6 (17%)	5 (14%)	10 (29%)	
Hispanic	11 (31%)	3 (9%)	11 (31%)	0.029
BMI (kg/m^2)	28 (25, 36)	29 (26, 33)	25 (22, 30)	0.036
Diabetes	5 (14%)	11 (31%)	11 (31%)	0.20
Hypertension	12 (34%)	12 (34%)	14 (40%)	0.90
Ritonavir use	13 (37%)	26 (74%)	24 (69%)	0.0040
Duration of prior TDF exposure (years)	1.5 (1.0, 2.0)	1.0 (0.5, 1.5)	1.5 (0.5, 2.0)	0.071
Current CD4$^+$ cell count (cells/μl)	412 (240, 593)	374 (263, 644)	306 (200, 502)	0.097
Nadir CD4$^+$ cell count (cells/μl)	188 (119, 318)	214 (108, 297)	159 (25, 211)	0.099
Detectable HIV viral load	12 (34%)	9 (26%)	16 (46%)	0.24
Baseline eGFRcr (ml/min per 1.73 m^2)	104 (93, 122)	97 (85, 108)	78 (61, 104)	<0.0001
Years of eGFRcr follow-up	7.3 (6.4, 9.1)	7.7 (7.3, 8.0)	7.0 (4.0, 7.8)	0.014

Continuous parameters are presented as median (IQR) and categorical parameters as n (%). AUC, area under the time concentration curve; eGFRcr, estimated glomerular filtration rate by creatinine; IQR, interquartile range; TDF, tenofovir disoproxil fumarate; TFV, tenofovir.

Table 2

Association of tenofovir area under the time concentration curve with annual change in estimated glomerular filtration rate by creatinine (ml/min/1.73 m^2) stratified by study follow-up interval (first 3.5 and second 3.5 years).

Parameter time period	TFV AUC tertile 1	TFV AUC tertile 2	TFV AUC tertile 3
Annual change in eGFR (95% CI):			
Year 0–3.5	1.63 (0.51, 2.7), P = 0.0044	1.68 (0.34, 3.0), P = 0.014	–0.24 (–1.49, 1.02), P = 0.71
Year 3.5–7.0	–1.40 (–2.4, –0.45), P = 0.0044	–2.4 (–3.7, –1.12), P = 0.0003	–1.92 (–3.2, –0.60), P = 0.0051
Unadjusted difference in annual change vs. tertile 1 (95% CI):			
Year 0–3.5	Reference	0.048 (–1.69, 1.78), P = 0.96	–1.87 (–3.5, –0.20), P = 0.029
Year 3.5–7.0	Reference	–0.99 (–2.6, 0.58), P = 0.22	–0.52 (–2.1, 1.10), P = 0.53
Adjusted[a] difference in annual change vs. tertile 1 (95% CI):			
Year 0–3.5	Reference	–1.41 (–3.2, 0.37), P = 0.12	–2.5 (–4.2, –0.76), P = 0.0046
Year 3.5–7.0	Reference	0.0079 (–1.61, 1.63), P = 0.99	0.17 (–1.56, 1.89), P = 0.85

Estimates from linear mixed models. AUC, area-under-the-time-concentration-curve; T1, TFV AUC tertile 1; T2, TFV AUC tertile 2; T3, TFV AUC tertile 3; TFV, tenofovir.

[a] Covariates in adjusted model include: age, race, BMI, diabetes mellitus, hypertension, ritonavir use, duration of prior tenofovir exposure, CD4+ cell count and HIV viral load.