

Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study

Christoph A Fux^{1*}, Mathew Simcock^{2,3†}, Marcel Wolbers², Heiner C Bucher^{2,3}, Bernard Hirschel⁴, Milos Opravil⁵, Pietro Vernazza⁶, Matthias Cavassini⁷, Enos Bernasconi⁸, Luigia Elzi³, Hansjakob Furrer¹ and the Swiss HIV Cohort Study

¹Division of Infectious Diseases, University Hospital Berne, Berne, Switzerland

²Basel Institute for Clinical Epidemiology, Basel, Switzerland

³Division of Infectious Diseases, University Hospital Basel, Basel, Switzerland

⁴Geneva University Hospital, Geneva, Switzerland

⁵University Hospital Zürich, Zürich, Switzerland

⁶Kantonsspital St Gallen, St Gallen, Switzerland

⁷Centre Universitaire Hospitalier Vaudois, Lausanne, Switzerland

⁸Hospital Civico Lugano, Lugano, Switzerland

†These authors contributed equally to this work

*Corresponding author: Tel: +41 31 632 27 45; Fax: +41 31 632 31 76; E-mail: christoph.fux@insel.ch

Background: A growing number of case reports have described tenofovir (TDF)-related proximal renal tubulopathy and impaired calculated glomerular filtration rates (cGFR). We assessed TDF-associated changes in cGFR in a large observational HIV cohort.

Methods: We compared treatment-naïve patients or patients with treatment interruptions ≥ 12 months starting either a TDF-based combination antiretroviral therapy (cART) ($n=363$) or a TDF-sparing regime ($n=715$). The predefined primary endpoint was the time to a 10 ml/min reduction in cGFR, based on the Cockcroft–Gault equation, confirmed by a follow-up measurement at least 1 month later. In sensitivity analyses, secondary endpoints including calculations based on the modified diet in renal disease (MDRD) formula were considered. Endpoints were modelled using pre-specified covariates in a multiple Cox proportional hazards model.

Results: Two-year event-free probabilities were 0.65 (95% confidence interval [CI] 0.58–0.72) and 0.80 (95% CI 0.76–0.83) for patients starting TDF-containing or TDF-sparing cART, respectively. In the multiple Cox model, diabetes mellitus (hazard ratio [HR]=2.34 [95% CI 1.24–4.42]), higher baseline cGFR (HR=1.03 [95% CI 1.02–1.04] by 10 ml/min), TDF use (HR=1.84 [95% CI 1.35–2.51]) and boosted protease inhibitor use (HR=1.71 [95% CI 1.30–2.24]) significantly increased the risk for reaching the primary endpoint. Sensitivity analyses showed high consistency.

Conclusion: There is consistent evidence for a significant reduction in cGFR associated with TDF use in HIV-infected patients. Our findings call for a strict monitoring of renal function in long-term TDF users with tests that distinguish between glomerular dysfunction and proximal renal tubulopathy, a known adverse effect of TDF.

Introduction

Tenofovir (TDF), a nucleotide reverse transcriptase inhibitor, has become a key component in combined antiretroviral therapy (cART) due to its favourable efficacy and safety profile [1]. In randomized, controlled clinical trials comparing TDF-based regimens against different standard regimens, no relevant side effects were noted; in particular, no renal safety signals were detected [2,3]. In the post-marketing era, however, a growing number of case reports have described TDF-related proximal renal tubulopathy,

including episodes with Fanconi syndrome [4–9]. TDF is partially eliminated via the proximal tubule where it is thought to accumulate, inducing excessive renal substance losses through impaired re-absorption after glomerular filtration [8–10]. Also, several studies have associated TDF medication with impaired glomerular filtration as evidenced by a lowered creatinine clearance [6,7,11,12]. This association, however, remains pathophysiologically unexplained, as no direct effect of TDF on the glomerular function has been documented.

This study was performed to gain more information about the effect of TDF use on the calculated glomerular filtration rate (cGFR) in a large HIV cohort. We analysed the frequency of reaching a sustained reduction in cGFR in patients initiating or restarting cART both with and without TDF, and characterized risk factors for a deterioration of cGFR.

Patients and methods

This study was performed in the context of the Swiss HIV Cohort Study (SHCS) (www.shcs.ch), which prospectively enrolls HIV-infected adults throughout Switzerland. Clinical and laboratory data are collected according to a standardized protocol at registration and follow-up visits every 6 months.

Study population

We identified all patients within the SHCS database, updated in February 2007, who started cART, defined as at least three antiretroviral drugs, whilst being either antiretroviral-naïve or untreated for ≥ 12 months. Patients had to have a baseline cGFR and at least two cGFR values while continuing either a TDF-containing or TDF-sparing cART regimen. The baseline cGFR was defined as the geometric mean of all cGFR measurements within 6 months prior to the (re)initiation of cART. To analyse the effect of TDF co-medication on cGFR, we defined four treatment groups for comparison: treatment-naïve and pre-treated patients starting cART either with TDF or without TDF, respectively.

Study evaluations

cGFR estimates were calculated based on the Cockcroft–Gault (CG) equation [13]: $([140 - \text{age (years)}] \times \text{weight [kg]} [\times 0.85 \text{ if female}]) / (72 \times \text{serum creatinine [mg/dl]})$. We preferred the CG equation to the modification of diet in renal disease (MDRD) equation as it enabled us to correct for weight changes during the study period. The closest weight within 6 months of the serum creatinine value date was used for the calculation; creatinine values without concomitant body weights were not included in the analysis.

The primary endpoint was the time to a 10 ml/min loss of cGFR. The reduction had to be maintained over ≥ 1 month during follow up in order to avoid misinterpretations of single estimations of cGFR. Patients without an event were censored at their last cGFR value while receiving the TDF-containing or TDF-sparing cART regimen.

Predefined covariates were age, gender, weight, ethnicity (Caucasian versus other), smoking status, diabetes mellitus, systolic blood pressure, baseline cGFR, HIV-1 plasma RNA, CD4⁺ T-cell count, AIDS status at baseline, and the use of boosted protease

inhibitors (PIs), cotrimoxazole as well as didanosine combined with TDF. Diabetes mellitus was defined as a fasting plasma glucose of 7 mmol/l, a casual plasma glucose > 11.1 mmol/l or current antidiabetic treatment. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg ($\geq 135/85$ mmHg in diabetic patients) or prescription of antihypertensive drugs.

In sensitivity analyses, the following secondary endpoints were analysed: drops in CG cGFR of 20 and 30 ml/min as well as drops of 10, 20 and 30%. In addition, we used the MDRD equation [14] to analyse the same endpoints: $(186 \times [\text{serum creatinine (mg/dl)}]^{-1.154} \times [\text{age (years)}]^{-0.203} [\times 0.742 \text{ if female}] [\times 1.212 \text{ if African}])$. We also calculated absolute changes in CG cGFR between baseline and 6, 12 and 24 months after (re)initiation of cART for the four patient groups. To derive the cGFR at these follow-up time points, the geometric mean of all available cGFR values in the interval ± 3 months of the respective time point was taken.

Data analysis

Kaplan–Meier estimates and plots of the predefined endpoints were produced for each of the four treatment groups. Patients receiving or not receiving TDF were compared overall and in the subgroups of treatment-naïve and pre-treated patients using the log-rank test. In a second step, data for the 10 ml/min and the 10% reduction in cGFR were modelled using the pre-specified covariates in a multiple Cox proportional hazards model. Interactions between TDF and boosted PI usage were also examined. For the other secondary endpoints no Cox regression analyses were performed due to low numbers of events.

Analyses were done using SAS, version 9.1.3 (SAS Institute, Cary, NC, USA). All reported confidence intervals (CIs) are two-sided 95% CIs and tests were performed at the two-sided 5% level.

Results

Patient characteristics

A total of 1,078 patients were included: 183 treatment-naïve patients starting TDF-containing cART (group 1), 180 patients reinitiating TDF-containing cART (group 2), 515 treatment-naïve patients starting TDF-sparing cART (group 3) and 200 patients reinitiating a TDF-sparing cART (group 4). Baseline characteristics of the four treatment groups are shown in Table 1. The median follow-up time was 19.8 months; follow up was significantly shorter in the TDF-treated groups, which may partly be explained by the fact that TDF was only introduced in 2002. Treatment-naïve patients were on average 2 years younger (39 compared to 41 years) and

Table 1. Baseline characteristics of the four treatment groups at the time of cART (re-)initiation

Factor	Treatment group			
	Naive to TDF-containing cART	Reinitiating TDF-containing cART	Naive to TDF-sparing cART	Reinitiating TDF-sparing cART
Patients, <i>n</i>	183	180	515	200
Median follow-up time, months (IQR)	14 (9, 21)	18 (11, 26)	23 (15, 35)	18 (11, 31)
Median time of (re)initiation of cART (IQR)	Mar 2005 (Jun 2004, Sep 2005)	Oct 2004 (Oct 2003, Jun 2005)	Oct 2003 (Nov 2002, Aug 2004)	Oct 2003 (Dec 2002, Oct 2004)
Demographics				
Median age, years (IQR)	39 (34, 47)	42 (37, 46)	39 (32, 45)	41 (36, 47)
Gender (female), <i>n</i> (%)	48 (26)	52 (29)	152 (30)	79 (40)
Median weight, kg (IQR)	69 (61, 79)	70 (60, 79)	69 (60, 78)	68 (59, 76)
Ethnicity (Caucasian), <i>n</i> (%)	145 (79)	164 (91)	398 (77)	177 (89)
Vascular risk factors				
Smoking, <i>n</i> (%)	88 (49)	86 (48)	217 (42)	123 (62)
Diabetes mellitus, <i>n</i> (%)	10 (5)	5 (3)	12 (2)	2 (1)
Arterial hypertension, <i>n</i> (%)	45 (25)	42 (23)	120 (23)	62 (31)
Median systolic bp, mmHg (IQR)	120 (110, 130)	120 (110, 128)	120 (110, 130)	120 (110, 132)
Median diastolic bp, mmHg (IQR)	80 (70, 85)	78 (70, 85)	80 (70, 85)	80 (70, 85)
HIV status				
Prior AIDS, <i>n</i> (%)	37 (20)	32 (18)	82 (16)	37 (19)
Median RNA, log ₁₀ copies/ml (IQR)	4.55 (3.44, 5.19)	4.76 (3.98, 5.23)	4.91 (4.12, 5.40)	4.85 (3.80, 5.30)
Median CD4 ⁺ T-cell count, cells/ μ l (IQR)	217 (152, 300)	225 (161, 277)	205 (125, 291)	235 (169, 325)
CD4 ⁺ T-cells <200 cells/ μ l, <i>n</i> (%)	77 (42)	68 (38)	249 (48.35)	75 (38)
Median CD4 ⁺ T-cell nadir, cells/ μ l (IQR)	204 (142, 270)	182 (122, 234)	188 (118, 259)	198 (122, 72)
Renal function				
Median serum creatinine, μ mol/l (IQR)	78.7 (67.0, 91.0)	78.4 (68.0, 87.1)	78.0 (67.0, 88.0)	76.0 (64.8, 86.0)
Median cGFR (MDRD) (IQR)	93.1 (82.4, 111.9)	95.2 (83.4, 09.0)	97.1 (85.2, 112.9)	94.8 (78.3, 114.0)
Median cGFR (CG) (IQR)	101.2 (83.9, 21.8)	100.12 (86.3, 118.0)	104.8 (89.5, 123.0)	104.5 (83.3, 21.1)
Co-medication				
Cotrimoxazol, <i>n</i> (%)	57 (31)	42 (23)	175 (34)	59 (30)
Didanosine, <i>n</i> (%)	20 (11)	48 (27)	23 (4)	32 (16)
Boosted PI, <i>n</i> (%)	73 (40)	101 (56)	208 (40)	80 (40)

Follow-up time: time from cART (re-)initiation until last available cGFR measurement while receiving the TDF-containing or TDF-sparing cART regimen. bp, blood pressure; cART, combination antiretroviral therapy, defined as at least three antiretroviral drugs; CG, Cockcroft–Gault formula (ml/min); cGFR, calculated glomerular filtration rate; IQR, interquartile range; MDRD, modification of diet in renal disease formula (ml/min per 1.73m²); PI, protease inhibitor; TDF, tenofovir.

less frequently Caucasian (78% compared to 90%). With 90% of the male but only 64% of the female patients being Caucasian, there was a significant association between gender and race ($P < 0.001$). The higher proportion of non-Caucasians among treatment-naive patients is possibly due to the increasing number of migrants in the SHCS over time [15]. Smoking and hypertension were more frequent in group 4 compared to groups 1–3 (62% versus 42–49% and 31% versus 23–25%, respectively), but median and quartiles of systolic and diastolic blood pressure were similar in the four groups. More treatment-naive patients had CD4⁺ T-cell counts <200 cells/ μ l and consequently were taking cotrimoxazole prophylaxis. By contrast, didanosine was more frequently used in treatment-experienced patients.

Event summary statistics

Therapy-naive and therapy-experienced patients starting TDF consistently showed reductions in CG cGFR between baseline and months 6, 12 and 24 (Table 2). Median changes from baseline at month 24 were -5.50 ml/min (interquartile range [IQR] -15.87, 7.85) and -4.11 ml/min (IQR -15.50, 4.71) in therapy-naive and pre-treated patients, respectively. In contrast, therapy-naive patients starting a TDF-sparing regime experienced a significant median increase in cGFR at all time points (median increase at 24 months was 2.68 ml/min [IQR -9.50, 13.76]), while no significant change occurred in patients reinitiating a TDF-sparing regime. Looking at the time-to-event analysis, 96 (26.4%) of the patients receiving TDF reached the

Table 2. Change from baseline in calculated glomerular filtration rates at 6, 12 and 24 months after (re)initiation of combined antiretroviral treatment both containing and sparing tenofovir

Treatment group	Change in cGFR (ml/min, according to Cockcroft-Gault formula) from baseline								
	at 6 months			at 12 months			at 24 months		
	<i>n</i>	Median (IQR)	<i>P</i> -value	<i>n</i>	Median (IQR)	<i>P</i> -value	<i>n</i>	Median (IQR)	<i>P</i> -value
Naive to TDF-containing cART	165	-4.5 (-11.6, 2.9)	0.0002	145	-2.8 (-10.6, 7.5)	0.06	54	-5.5 (-15.9, 7.9)	0.06
Reinitiating TDF-containing cART	163	-2.9 (-12.1, 4.5)	0.0006	143	-2.6 (-13.8, 4.4)	0.0008	84	-4.1 (-15.5, 4.7)	0.004
Naive to TDF-sparing cART	470	2.6 (-5.6, 11.6)	<0.0001	452	3.8 (-7.1, 15.1)	<0.0001	349	2.7 (-9.5, 13.8)	0.02
Reinitiating TDF-sparing cART	185	1.8 (-7.0, 9.9)	0.09	168	1.0 (-7.5, 9.4)	0.4	118	0.8 (-8.7, 9.1)	0.6

n refers to the number of patients with at least one cGFR within ± 3 months of the respective time point. *P*-values are based on Wilcoxon signed rank test. cART, combination antiretroviral therapy; cGFR, calculated glomerular filtration rate; IQR, interquartile range; TDF, tenofovir.

primary endpoint in 422.1 person-years of follow up (22.7 events/100 person-years, 95% CI 18.2–27.3), compared to 128 (17.9%) patients without TDF who reached an event in 1240.2 person-years of follow up (10.3 events/100 person-years, 95% CI 8.5–12.1). In the first and second year of cART administration, event rates per 100 person-years of follow up were 27.3 (95% CI 21.2–33.5) versus 14.5 (95% CI 11.5–17.6) and 14.0 (95% CI 6.9–21.1) versus 8.9 (95% CI 5.8–12.0) for patients on TDF-containing and TDF-sparing cART, respectively. Two-year event-free probability estimates for the primary endpoint were 0.60 (95% CI 0.47–0.72) in group 1, 0.67 (95% CI 0.58–0.75) in group 2, 0.80 (95% CI 0.76–0.83) in group 3 and 0.80 (95% CI 0.73–0.86) in group 4 (Figure 1). The time to a sustained reduction of cGFR was significantly shorter for patients treated with TDF (groups 1 and 2) than for patients not taking TDF (groups 3 and 4) (log-rank test; $P < 0.0001$). These differences between TDF-containing and TDF-sparing regimens were also significant in the subgroups of treatment-naïve and pre-treated patients ($P = 0.0003$ and 0.02 , respectively). Event rates were similar and conclusions highly consistent when a sustained drop of 10% was considered or calculations were based on the MDRD formula instead (data not shown).

Two-year event-free probability estimates for a sustained drop by 20 ml/min in CG-based cGFR were 0.84 (95% CI 0.73–0.91) in group 1, 0.83 (95% CI 0.74–0.89) in group 2, 0.91 (95% CI 0.88–0.93) in group 3 and 0.94 (95% CI 0.90–0.97) in group 4. Again, the time to an event was significantly shorter in the TDF-containing groups ($P = 0.008$) and the same was true when a sustained 20% reduction was considered instead ($P = 0.0002$).

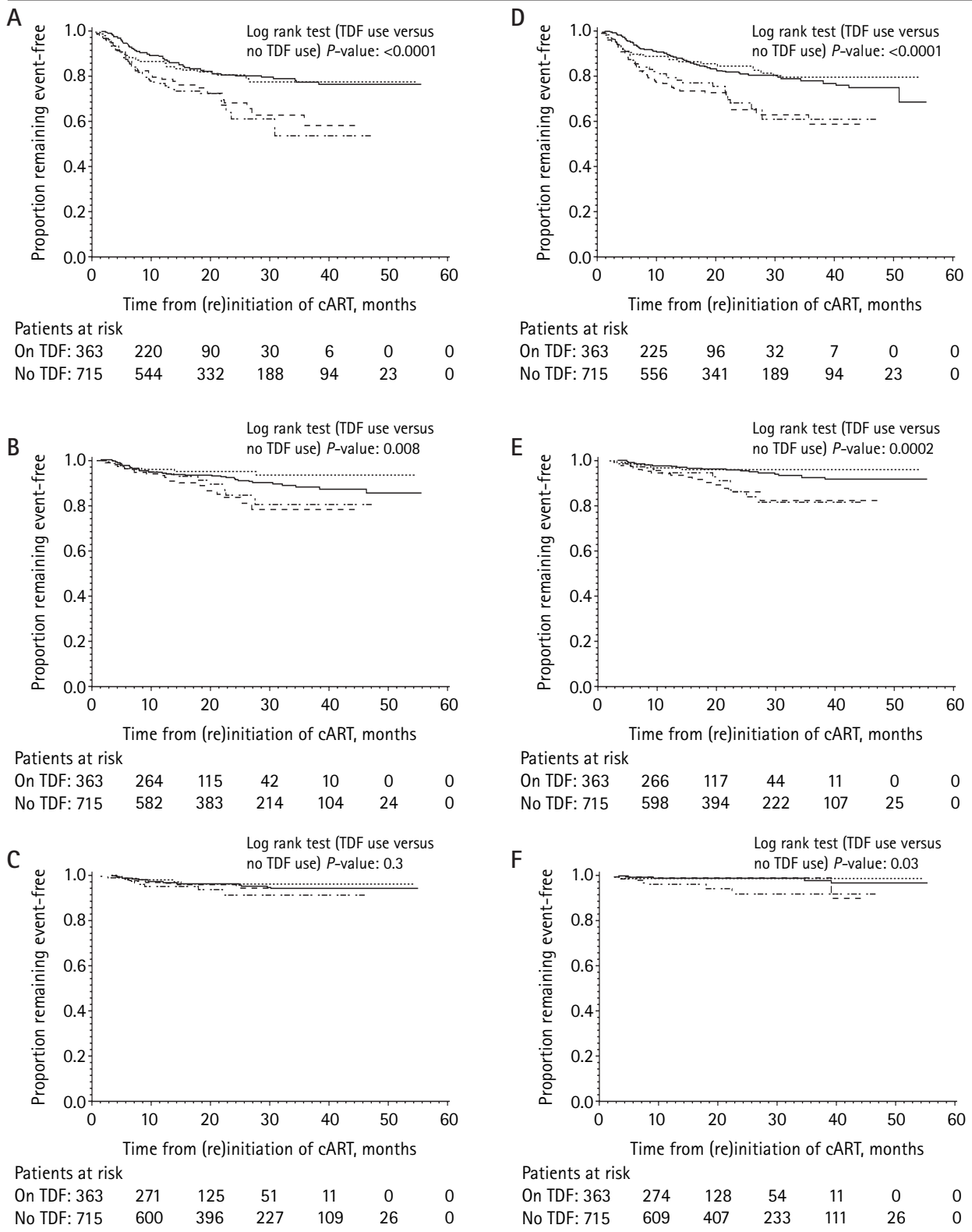
In the 224 patients reaching the primary endpoint, the median duration of sustained cART reduction was

9.1 (IQR 5.8, 17.9) months, without significant differences between the four groups studied ($P = 0.2$): treatment-naïve patients initiating cART+TDF 10.2 (IQR 5.4, 16.1) months; patients reinitiating cART+TDF 7.9 (IQR 5.9, 17.6) months; treatment-naïve patients initiating TDF-sparing cART 11.4 (IQR 6.1, 23.1) months; and patients reinitiating TDF-sparing cART 8.7 (IQR 3.8, 15.9) months.

Multiple Cox regression analysis

Results from the multiple Cox regression to predict the time to a sustained 10 ml/min reduction of CG cGFR are shown in Table 3 in the first column. Female gender (hazard ratio [HR]=1.62 [95% CI 1.17–2.25]), diabetes mellitus (HR=2.34 [95% CI 1.24–4.42]), TDF use (HR=1.84 [95% CI 1.35–2.51]), co-medication with boosted PIs (HR=1.71 [95% CI 1.30–2.24]) and baseline cGFR (HR=1.03 [95% CI 1.02–1.04]) significantly increased the risk of reaching the primary endpoint. When the subgroups of patients with and without boosted PIs were analysed separately, the hazard ratio associated with TDF usage was higher for patients on boosted PIs (HR=2.87 [95% CI 1.80, 4.60]) compared with unboosted PIs (HR=1.52 [95% CI 0.97, 2.4; $P = 0.07$]). An interaction term between TDF and boosted PI usage in the overall analysis was non-significant ($P = 0.2$). The results of the major sensitivity analyses, the 10% reduction in CG cGFR and analyses based on the MDRD equation are also depicted in Table 3. TDF was significantly associated with a higher hazard of declining cGFR in all these analyses, although the effect was somewhat smaller using the MDRD equation. While most covariates were consistent across the four treatment groups, there were some discrepancies: only the gender effect was significant in CG-based calculations; systolic blood pressure and weight were found to exclusively affect

Figure 1. Kaplan–Meier plots for time to a sustained reduction in calculated glomerular filtration rates according to treatment experience and tenofovir use



Kaplan–Meier plots for time to a sustained absolute reduction in calculated glomerular filtration rate by (A) 10, (B) 20 and (C) 30 ml/min and a sustained relative reduction by (D) 10, (E) 20 and (F) 30% are shown. Glomerular filtration rates were calculated according to the Cockcroft–Gault formula. Group 1 (mixed line): treatment-naïve patients initiating tenofovir (TDF)-containing combined antiretroviral treatment (cART). Group 2 (dashed line): treatment-experienced patients initiating TDF-containing cART. Group 3 (solid line): treatment-naïve patients initiating TDF-sparing cART. Group 4 (dotted line): treatment-experienced patients initiating TDF-sparing cART. Log-rank *P*-values represent the comparison between those patients (re)initiating a TDF-containing cART versus those (re)initiating a TDF-sparing cART.

the risk of a 10 ml/min reduction in the MDRD-derived cGFR, whereas viral load only affected the CG-based reduction by 10%. All other covariates, including age, smoking status, CD4⁺ T-cell count, previous exposure to ART and usage of cotrimoxazole did not significantly influence the hazard of reaching the defined endpoints in the primary analysis or sensitivity analyses.

Discussion

cGFR in tenofovir-treated patients

In the TDF treatment groups we found a modest, but consistent, decrease in median cGFR between baseline and months 6, 12 and 24. By contrast, median cGFR remained stable in patients reinitiating a TDF-sparing regime and even increased in the group of patients initiating a first cART without TDF. These findings are congruent with a longitudinal analysis that described a decrease in cGFR from 102 to 94.5 ml/min in TDF-treated patients, but not in controls [16].

From a methodological point of view, the analysis of median values could miss important deteriorations in cGFR, if only a subpopulation was affected by TDF toxicity. Furthermore, measurements at predefined time points, such as the last follow up available

[17,18], have limitations and could compromise the sensitivity to detect deteriorations in cGFR. When we used a time-to-event endpoint instead, we found an almost twofold increased risk of reaching a sustained 10 ml/min reduction in CG cGFR in patients starting TDF compared with patients receiving a TDF-sparing cART. The higher risk for cGFR deterioration associated with TDF use was not restricted to the primary endpoint, but was consistently shown in the sensitivity analyses such as cGFR reductions by 20 ml/min as well as 10 and 20% relative reductions, irrespective of the cGFR formula type used and the pre-treatment status (naive or experienced). This consistency provides strong evidence for a significant negative effect of TDF on the cGFR in an important subset of HIV-infected patients.

The decision to define the primary endpoint as the sustained decline in CG cGFR of 10 ml/min was based on data from chronic diabetic, hypertensive or non-diabetic proteinuric kidney diseases, where a similar reduction per year is an ominous sign of progressing nephropathy. In fact, the current aim in the treatment of these pathologies is to limit the decrease in GFR to 1–2 ml/min per year [19].

The restriction to sustained cGFR reductions in our analyses is unique. With 9.1 (IQR 5.8–17.9) months,

Table 3. Multivariable Cox regression analysis for the time to a sustained reduction in cGFR

Factor	cGFR reduction of 10 ml/min by CG		cGFR reduction of 10 ml/min by MDRD		cGFR reduction of 10% by CG		cGFR reduction of 10% by MDRD	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (by +10 years)	0.89 (0.77–1.04)	0.1	0.97 (0.84–1.11)	0.6	1.03 (0.89–1.20)	0.7	1.01 (0.88–1.16)	0.9
Female gender	1.62 (1.17–2.25)	0.004	1.05 (0.77–1.42)	0.8	1.784 (1.29, 2.47)	0.0005	1.28 (0.95–1.73)	0.1
Weight (by +10 kg)	1.10 (0.99–1.23)	0.08	0.87 (0.78–0.98)	0.02	1.04 (0.93–1.16)	0.5	0.93 (0.83–1.03)	0.2
Non-Caucasian ethnicity	0.99 (0.66–1.47)	0.9	1.31 (0.92–1.85)	0.1	0.91 (0.61–1.38)	0.7	1.00 (0.69–1.44)	0.99
Smoking	1.22 (0.92–1.61)	0.2	1.09 (0.84–1.41)	0.5	1.18 (0.89–1.56)	0.3	1.11 (0.86–1.43)	0.42
Diabetes mellitus	2.34 (1.24–4.42)	0.008	1.82 (0.97–3.41)	0.06	2.38 (1.25–4.50)	0.008	1.95 (1.04–3.66)	0.04
Systolic blood pressure (by +10 mmHg)	1.04 (0.95–1.14)	0.4	1.10 (1.02–1.19)	0.02	1.05 (0.95–1.145)	0.3	1.07 (0.99–1.16)	0.1
Baseline cGFR (by +10 ml/min)	1.03 (1.02–1.04)	<0.0001	1.02 (1.02–1.03)	<0.0001	1.03 (1.02–1.04)	<0.0001	1.02 (1.02–1.03)	<0.0001
RNA, log ₁₀ copies/ml	1.05 (0.94–1.18)	0.4	1.07 (0.97–1.19)	0.2	1.13 (1.00–1.27)	0.04	1.07 (0.97–1.19)	0.2
CD4 ⁺ T-cell count (by +100 cells/μl)	1.08 (0.98–1.19)	0.1	1.02 (0.93–1.13)	0.6	1.10 (0.99–1.212)	0.7	1.02 (0.92–1.13)	0.7
AIDS status	1.04 (0.72–1.51)	0.8	1.32 (0.97–1.79)	0.08	1.02 (0.70–1.48)	0.9	1.24 (0.91–1.69)	0.2
Previous drug exposure								
ART	0.97 (0.72–1.30)	0.8	1.02 (0.78–1.34)	0.9	0.88 (0.65–1.19)	0.4	1.04 (0.80–1.36)	0.8
TDF	1.84 (1.35–2.51)	0.0001	1.62 (1.22–2.14)	0.0008	2.01 (1.47–2.77)	<0.0001	1.67 (1.27–2.21)	0.0003
Didanosine with TDF	1.36 (0.85–2.17)	0.2	1.09 (0.69–1.72)	0.7	1.48 (0.93–2.36)	0.1	1.11 (0.71–1.74)	0.7
boosted PI	1.71 (1.30–2.24)	0.0001	1.88 (1.46–2.41)	<0.0001	1.74 (1.31–2.31)	0.0001	2.04 (1.59–2.62)	<0.0001
Cotrimoxazol	1.07 (0.76–1.52)	0.7	1.30 (0.96–1.75)	0.09	1.16 (0.82–1.65)	0.4	1.27 (0.94–1.71)	0.1

Multivariable Cox regression analysis for the time to a sustained reduction in the calculated glomerular filtration rate (cGFR) of 10 ml/min and 10%, respectively. The primary endpoint, the cGFR reduction of 10ml/min by CG, is displayed in the first column. ART, antiretroviral therapy; CG, Cockcroft-Gault formula; CI, confidence interval; MDRD, modification of diet in renal disease formula; PI, protease inhibitor; TDF, tenofovir.

the median duration of a sustained cGFR reduction exceeded the predefined minimum of 1 month by far. This persistence beyond the KDOQI criterion of 3 months to indicate chronic kidney disease excludes episodes of transient pre- or post-renal nephropathy with high certainty. Such episodes are unlikely to be related to TDF use, as has been shown in the Chelsea and Westminster cohort, where any recorded creatinine value $>120 \mu\text{mol/l}$ was analysed and TDF-independent etiologies of renal dysfunction were found in 90% of cases [20]. In the analyses of the Johns Hopkins cohort, the authors tried to lower the effect of TDF-independent acute episodes of nephropathy by averaging the maximum and the subsequent serum creatinine level occurring within 1 year of follow up [11] and found TDF use to be associated with a larger absolute (-13.3 ml/min versus -7.5 ml/min) and relative (-10% versus -6%) peak change in CG cGFR [11].

Two randomized, controlled trials showed that patients on TDF-sparing regimens experienced a modest increase in CG cGFR, while cGFR remained stable in patients on TDF [21,22]. In the latter study, this difference disappeared in MDRD-based calculations and at the 96-week time point [3]. This low rate of renal impairment in clinical trials, as opposed to observational studies, may be due to methodological differences, including the stringent inclusion criteria used in randomized, controlled trials excluding patients at high risk for deterioration of kidney function and the relatively short follow-up [21–24]. The stability of cGFR after TDF initiation of the Thai patients included in the Staccato trial could be explained with the selection of younger patients, the shorter follow-up and the comparison of mean cGFR values measured at predefined time points [25].

Cockcroft–Gault versus MDRD

We observed some discrepancies between the HRs calculated based on the CG and the MDRD formula (Table 3). This is not surprising as, although both formulas correct for gender and age, only the CG formula corrects for body weight and only the MDRD formula corrects for ethnicity. The use of the CG formula appeared preferable for our study, as the mean body weight increased by $>2 \text{ kg}$ between baseline and the time of the event/censoring ($P<0.0001$). Weight adjustments are intended to correct for differences in muscle mass, the source of creatinine. A disproportional increase in muscle mass in response to cART, as has been observed in patients with greater pre-treatment immunocompromise [26], could falsely associate AIDS status with treatment-related renal impairment in weight-unadjusted MDRD calculations ($P=0.08$ in our data), but not in weight-adjusted CG calculations ($P=0.8$ in our data; Table 3). Also, a low

muscle mass may mask negative effects of obesity on cGFR if the MDRD ($\text{HR}=0.87$ by $+10 \text{ kg}$ higher weight; $P=0.02$) instead of the CG equation ($\text{HR}=1.10$; $P=0.08$) are used. The observed differences in the HR according to gender between GC- ($P=0.004$) and MDRD-derived data ($P=0.1$) could also be related to differences in body composition. Clearly, they cannot be explained by an interaction between gender and race: both Caucasian and non-Caucasian females had a significantly higher risk of achieving the endpoint in the CG- but not the MDRD-based formula.

Importantly, the HRs associated with TDF and boosted PI use showed high consistency between the GC- and MDRD-based calculations.

Associated risk factors

In our study, boosted PI usage was an independent risk factor for reaching the primary endpoint. Some previous studies found an increased risk for reduced cGFR when combining TDF and boosted PIs [8,24,27,28]. To the best of our knowledge, TDF-independent nephrotoxicity of boosted PIs except for indinavir has not been described. The combined effect has been explained by PIs inhibiting the tubular multidrug resistance protein 2 (MRP2) transporter and/or the P-glycoprotein. The inhibition may lead to increased TDF concentrations in both the serum and the proximal tubular cell, thus increasing TDF toxicity [29]. However, no correlation between TDF plasma levels and cGFR was found in patients treated with boosted atazanavir [28]. Also, recent publications have claimed that TDF is not a substrate for MRP2, but for the PI-independent transporter MRP4 [30]. We found some evidence that the TDF effect was higher for patients on boosted PI, but no significant interaction between TDF and boosted PI usage ($P=0.2$). While interaction tests often have low power and should be interpreted with caution, this argues for a direct, TDF-independent compromise of cGFR by PIs, which should be addressed in more detail in further studies.

Diabetes mellitus was associated with a highly significant HR point estimate of 2.34 (95% CI 1.24–4.42) for a sustained reduction in cGFR. This indicates that, as suspected, diabetes mellitus is a clinically relevant factor. In contrast to the Johns Hopkins cohort, lower CD4⁺ T-cell counts at baseline were not associated with a decline in cGFR in our study, while the use of boosted PIs consistently was [11]. In previous studies, renal dysfunction has been related to mitochondrial DNA depletion, in particular in patients receiving concurrent TDF and didanosine, which has a toxic effect on the mitochondrion [31]. In our analysis, as well as in an earlier study by Gérard *et al.* [28], didanosine co-medication did not increase the risk for renal dysfunction.

Limitations

This study design has some important methodological limitations. First, the non-randomized allocation to a specific treatment has introduced systematic differences between the treatment groups. For example, patients reinitiating a TDF-sparing cART regimen were more likely to smoke or have arterial hypertension (Table 1). However, the observation that TDF had a deleterious effect in both cART-naïve and cART-experienced patients argues for an etiological role of TDF. Second, as the analysis is restricted to data available in the SHCS database, additional confounding factors, such as pre-renal causes of acute nephropathy like dehydration or sepsis, could not be entered into the analysis. Third, the follow up is still too short to verify whether the cumulative proportion of patients with impaired renal function parameters is further increasing over time, as suggested by the current analysis. Also, the reduction in cGFR does not prove glomerular dysfunction. As up to 35% of the urinary creatinine is derived from tubular secretion [32,33], a reduced creatinine clearance does not prove impaired glomerular filtration, but may indicate proximal renal tubulopathy, a well-known adverse effect of TDF. However, given the increase in patients affected by cGFR losses over time, it is unlikely that TDF is only inhibiting creatinine secretion at the tubular level as a simple competitor such as is known for sulphonamides or pyrimethamine [34].

Conclusions

The consistency in our data provides strong evidence for a negative effect of TDF on the cGFR, at least in a subpopulation of HIV-infected patients. For methodological reasons, reductions in cGFR do not prove glomerular dysfunction, but may indicate reduced tubular secretion of creatinine. If, however, the observed changes in cGFR correspond to a chronic and progressive reduction in glomerular filtration rates in a substantial proportion of treated patients, the nephrotoxicity of TDF-based cART would be unacceptably high. Studies with longer follow up and investigations that specifically assess tubular function and creatinine- (that is, tubulus-) independent measurements of GFR, such as ¹²⁵I-iothalamate or serum cystatin C levels [35,36], are needed. Also, the reversibility of cGFR impairment upon discontinuation of TDF that has been observed in most patients with drug-related Fanconi syndromes should be investigated.

In the meantime, clinicians should monitor patients on TDF for a decline in cGFR and evaluate proximal tubular function in affected patients by quantifying phosphataemia, the fractional excretions of phosphate and uric acid as well as normoglycaemic glucosuria. In case of proteinuria, the glomerular or tubular origin of proteins should be determined by electrophoresis.

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