ORIGINAL RESEARCH

Comparison of Renal Function Biomarkers of Serum Creatinine and Cystatin C in HIV-Infected People on Dolutegravir-Containing Therapy

Lianfeng Lu[1], Xiaodi Li[1], Xiaosheng Liu[2], Yang Han[1], Zhifeng Qiu[1], Xiaojing Song[1], Yanling Li[1], Xiaoxia Li[1], Wei Cao[1], Taisheng Li[1,2]

[1]Department of Infectious Diseases, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, People's Republic of China; [2]Tsinghua-Peking Center for Life Sciences, Beijing, China Department of Basic Medical Sciences, School of Medicine, Tsinghua University, Beijing, People's Republic of China

Correspondence: Taisheng Li, Department of Infectious Diseases, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, 1# Shuai Fu Yuan, Beijing, 100730, People's Republic of China, Email litsh@263.net

Objective(s): HIV-1-infected Chinese patients who were treated naïve with combination dolutegravir (DTG) and tenofovir disoproxil fumarate (TDF) group, DTG without TDF group, TDF without DTG, as well as patients switched to DTG-containing therapy from other drugs were included.

Design: The dynamics of serum creatinine, cystatin C (CysC) level, $eGFR_{cr}$ and $eGFR_{CysC}$ at the baseline, 4 w, 12w, 24w, 36w and 48w for different group of patients were collected and evaluated.

Methods: Changes in serum creatinine, levels, $eGFR_{cr}$ and $eGFR_{CysC}$ were analyzed among groups and in different time-points. Intra-group correlation coefficient and Bland–Altman plot were used to compare the results of $eGFR_{cr}$ and $eGFR_{CysC}$.

Results: Thirty-seven treated-naïve HIV-patients in combined DTG and TDF group (group 1), 23 in DTG without TDF patients (group 2) and 47 patients on TDF without DTG group (control group, group 3) along with 31 patients whose ART switch to DTG-containing regimens (group 4) were collected. Serum creatinine was significantly elevated in the group 1 and group 2 instead of group 3 from baseline to 48w. Mean decreased change of eGFR calculated by serum creatinine proved the same conclusion. However, there were no differences in serum cystatin C and $eGFR_{CysC}$ between baseline and at 48 weeks in DTG-containing groups. Moreover, the proportion of eGFRcr decreased over 30% was significantly higher in DTG-treatment group.

Conclusion: We demonstrated the clinical benefits of CysC for assessing the glomerular filtration rate when evaluating renal function in HIV-1-infected patients treated with whether DTG combined with TDF or not.

Keywords: HIV, antiretroviral therapy, dolutegravir, renal function, cystatin C, serum creatinine

Introduction

With the advent of antiretroviral therapy (ART) has contributed to protracted survival in patients infected with human immunodeficiency virus 1 (HIV-1), comorbidities with aging and side effects of ART have emerged as new concerns. Early detection of kidney damage is important in selecting appropriate antiretroviral drugs and eliminating potential nephrotoxic drugs for people infected with HIV-1. Tenofovir (TDF) is an important nucleotide reverse transcriptase inhibitor (NRTIs) combined with Lamivudine (3TC) and Efavirenz (EFV) as a first-line treatment for HIV-1-infected patients in China.[1] One of the adverse effects of TDF is acute or chronic kidney injury which reported approximately 4–8%, usually occurred within 1 year.[2–4] However, in some long-term follow-up studies, only a mild decline in eGFR can be attributed to TDF[5,6] while others believed TDF can cause renal damage in long-term.[7,8]

Recently, DTG(dolutegravir) has been promoted as a newer integrase inhibitor drug, which may inhibit renal creatinine secretion by inhibiting some transporters, including the organic cation transporter 2 and multidrug and toxin extrusion 1, causing a slight increase in serum creatinine level, whereas there were rarely reports of actual renal damage.[9,10] Combined

DTG regimen or single-tablet regimen containing DTG will increase 10–20% serum creatinine from baseline in randomized clinical trials.[9,11,12] Thus, differentiating the cause and clinical significance of increases in serum creatinine may be challenging even though combined TDF and DTG are effective in inhibiting HIV viral replication.

Serum creatinine (Cre) is the most common and easiest clinical marker for assessing kidney function, but it is often affected by age, gender, weight and muscle mass.[13] As dolutegravir inhibits renal creatinine secretion, an increase in serum creatinine may be observed in HIV-1-infected individuals treated with dolutegravir, serum creatinine-based estimated GFR (eGFR$_{cr}$) may be lower than actual GFR. Serum cystatin C can be another marker for estimation of GFR(eGFR$_{CysC}$), which is produced by nucleated cells at a constant production rate, freely filtered without tubular secretion or reabsorption and catabolized in the proximal tubule, but eGFR$_{CysC}$ may be affected by HIV status including viraemia.[14]

Overall, it is important to describe the impact of dolutegravir on renal function beyond the controlled clinical trial environment to verify the safety profile of INSTIs and potential renal damage in HIV-infected patients. The rate and significance of increased serum creatinine out of clinical trials and in Chinese HIV population were unknown. In this study, we compared the serum creatinine elevation between combination with DTG and TDF group, DTG without TDF group and TDF without DTG group for control. We also examined the utility of different biomarkers: serum creatinine and cystatin C of kidney function in people infected with HIV-1 treated with dolutegravir.

Methods

Study Design and Data Sources

The medical records for a single HIV-1 patient center at the AIDS Clinical Center, Peking Union Medical Hospital, were examined retrospectively. HIV-positive patients older than 18 years were enrolled irrespective of sex whereas patients younger than 18 years, those who had no records of serum creatinine, who had severe neuropathy or renal transplantation were excluded. The change of serum creatinine and Cystatin C was investigated on treatment-naïve patients who started, DTG combined TDF therapy (group 1), DTG-containing without TDF ART (group 2) and those who started TDF-containing without DTG ART (group 3) as the control, and patients who switch-to DTG-containing ART patients (group 4). In the first year after commencing antiretroviral therapy, patients regularly visit clinic at 4, 12, 24, 36 and 48 weeks. After the first year, patients usually visited our clinic at least every 3 months for monitoring CD4+ cell count, HIV-1 viral load, and other laboratory tests including serum creatinine, ALT and AST, whole blood test, blood sugar level, urinary routine test. Cystatin C was not a regular test included in every patient but examined according to clinical needs.

For related risk factors, we included age, sex, body weight, history of AIDS, route of HIV-1 transmission, baseline laboratory data (CD4+T cell count, HIV viral load, and serum creatinine, urinary routine test), and presence or absence of other Medical conditions (concurrent use of ritonavir-boosted PIs (PI/r), concurrent nephrotoxic drugs such as ganciclovir and sulfamethoxazole/trimethoprim, diabetes mellitus defined by using antidiabetic agents or fasting plasma glucose higher than 126 mg/dl or plasma glucose higher than 200 mg/dl on two different days, hypertension defined by current treatment with antihypertensive agents or two successive measurements of systolic blood pressure (SBP) higher than 140 mmHg or diastolic blood pressure(DBP) higher than 90 mmHg at the clinic, coinfection with hepatitis B defined by positive hepatitis B surface antigen, coinfection with hepatitis C defined by positive HCV-Ab or positive HCV-RNA).

Laboratory Methods and Calculation

All of laboratory tests were routinely finished in our hospitals. Serum creatinine was evaluated in Beckman Coulter reagents used on AU5800 analyzers in clinical laboratories of Peking Union Medical College Hospital. Cystatin C(CysC) was assayed by particle-enhanced turbidimetric assay (PETIA; Beijing Strong Biotechnologies, Inc., Beijing, China). The plasma HIV-1 RNA load was measured using the COBAS Ampliprep/TaqMan 48 real-time RT-PCR Test (Roche, CA, USA) according to the manufacturer's instructions. Immune profile of peripheral blood lymphocytes was analyzed by three-color flow cytometry (Epics XL flow cytometry; Beckman Coulter, USA).

eGFR was calculated using the CKD-EPI Chinese equation based on standardized serum creatinine, sex, and age,[15] but also GFR based on CysC was calculated.[16]

The equation of GFR calculation was listed as following:

①CKD-EPI$_{scr}$: eGFR=ax(Scr/b)^{c}x(0.993)^{age}

a: If the patient is black: male:163, female:166; If the patient is not black: male:141, female:144

b: male:0.9; female:0.7; c: (1) female:Scr≤0.7mg/dl, −0.329; Scr>0.7mg/dl,-1.209; (2) male:Scr≤0.9mg/dL,-0.411; Scr>0.9mg/dL,-1.209

②CKD-EPI$_{SCysC}$:eGFR=133x(S$_{CysC}$/0.8)^{a}x(0.996)^{age}x(0.932, if female)

a:S$_{CysC}$≤0.8mg/L,-0.499; S$_{CysC}$>0.8mg/L,-1.328;

The baseline eGFR was estimated for each patient from age, sex, and serum creatinine measurements closest to and preceding the commencement of ART by no more than±14 days. The main consequences were serum creatinine and CysC level change from baseline, 4w, 12w, 24w, 36w till 48 weeks. If observed more than 30% decrement in eGFR relative to the baseline or eGFR lower than 60 mL/min per 1.73 m2, the clinical doctors would change potential nephrotoxic drugs.

Statistical Analysis

Statistical analysis was performed using SPSS software (SPSS® for Windows™ version 22.0, SPSS Inc., Chicago, IL, USA), GraphPad Prism 8.0 (GraphPad Software, Inc. La Jolla, CA, USA) and MedCalc v19.0.4. Data distribution was examined by Kolmogorov–Smirnov test. Reference ranges were presented using mean ± 2 standard deviation for parametric data and 2.5% and 97.5% percentages for non-parametric data. Comparisons among three variables were performed using one-way analysis of variance, while t-test for two groups parametric data and Mann–Whitney test for non-parametric data. Change in creatinine, CysC, eGFR were plotted from the baseline measurement until occurrence of change ART combination before 48 week or until 48 weeks. The effects of treatment and time on eGFR were evaluated using a mixed linear model. In this study, intra-group correlation coefficient, and Bland–Altman were used to compare the results of eGFR$_{cr}$ and eGFR$_{CysC}$ from one collection simultaneously measured. A P-value of less than 0.05 was considered statistically significant.

Results

Basic Characteristics at Enrollment

The characteristics of the patients are shown in Table 1. Total of patients were prescribed dolutegravir, including 37 patients were treated-naïve DTG+TDF-containing (group 1),23 patients treated with DTG-containing but no TDF (group 2) and 31 patients were switched-to DTG (group 4). And 47 patients using 3TC+TDF+EFV were picked up as control group (group 3) which was the first-line treatment in China. In general, the mean age was 36.73±11.32 years, the mean weight was 68.19±10.48 kg. One hundred and thirty (94.2%) patients were male, and eight (5.8%) patients were female. There were no significant differences between the treated-naïve groups with respect to baseline serum creatinine, cystatin C, eGFR$_{cr}$, eGFR$_{CysC,}$ age, sex, weight, route of transmission, HBV and HCV infection, basic urine routine tests and status of diabetes, hypertension as well as co-current nephrotoxic drugs, only significant differences were detected regarding baseline CD4+T-cell level (group 1 versus group 2 versus group 3: p=0.004.)as well as opportunistic infections (group 1 versus group 2 versus group 3: p=0.018). Considering switch-to DTG group, only the rate of the transmission route was different from control group (group 3 vs group 4: p=0.017). The baseline serum creatinine, cystatin C, eGFR$_{cr}$, eGFR$_{CysC,}$ and other factors were balanced in group 3 and group 4. Considering key drugs before switching to dolutegravir, usage of NNRTIs was 38.7% (n =12), PIs was 22.5% (n = 7), and INSTIs was32.3% (n = 10). Among them, the INSTI-based ART in 10 patients was totally Raltegravir(RAL), 7 patients using PI-based ART were Lopinavir and ritonavir (LPV/r).

Table 1 Baseline Characteristics of These Patients Among Four Groups

Variables	Combined DTG and TDF(n=37) Group1	DTG Without TDF(n=23) Group 2	TDF Without DTG (n=47) Group 3	Switch-to DTG(n=31) Group 4	P-Value†	P-Value‡
Age, mean years	38.9±12.6	40.2±15.7	34.3±9.2	34.8±8.1	0.128	0.803
Male, n(%)	35(94.6)	23(100)	44(93.6)	29(93.5)	0.714	0.677
Route of transmission, n(%)						
Homosexual	23(62.2)	17(73.9)	35(74.5)	16(51.6)	0.784	0.017
Heterosexual	6(16.2)	2(8.7)	5(10.6)	2(6.5)		
Blood	0(0.0)	0(0.0)	0(0.0)	4(12.9)		
Unclear/others	8(21.6)	4(17.4)	7(14.9)	9(29.0)		
Viral load (lg copies/mL)	5.16±0.77	4.76±0.68	4.80±0.80	4.66±0.67	0.007	0.423
CD4+T cell(cell/ul)	192(78,344)	372(183,631)	257(118,467)	304(194,417)	0.004	0.672
Opportunistic infection, n(%)	12(32.4)	8(34.8)	5(10.6)	7(22.6)	0.018	0.068
HBsAg(+),n(%)	2(5.4)	0(0.0)	3(6.4)	1(3.2)	0.714	0.648
HCV-Ab(+),n(%)	2(5.4)	0(0.0)	0(0.0)	2(6.5)	0.215	0.059
Type 2 Diabetes, n(%)	5(13.5)	1(4.3)	2(4.3)	5(16.1)	0.252	0.107
Hypertension, n(%)	5(13.5)	2(8.7)	2(4.3)	1(3.2)	0.355	0.654
Initial ART regimen, n(%)	NA	NA				
2NRTIs + NNRTI			47(100)	12(38.7)		
2NRTIs+PI				7(22.5)	NA	NA
2NRTIs + INSTI				10(32.3)		
Other				2(6.5)		
Nephrotoxic drug, n(%)	9(24.3)	3(13.0)	15(31.9)	4(12.9)	0.419	0.255
Weight(kg)	66.6±8.20	66.4±14.16	70.8±7.65	67.6±1.84	0.892	0.127
Urine test abnormality, n(%)	3(8.1)	1(4.3)	3(6.4)	2(6.5)	0.895	0.648
Baseline serum creatine(umol/L)	75.5±13.4	77.2±12.4	77.1±10.7	82.9±12.9	0.806	0.062
Baseline Cystatin C(mg/L)	1.01±0.21	1.16±0.34	0.99±0.29	0.86±0.14	0.255	0.102
Baseline eGFR$_{cr}$(ml/min per 1.73 m2)	107.7±12.7	105.3±13.9	114.5±17.1	106.3±18.7	0.070	0.051
Baseline eGFR$_{Cysc}$(ml/min per 1.73 m2)	87.09±23.6	77.3±31.5	93.2±27.4	104.9±18.5	0.368	0.128

Notes: P-value†: comparison among group 1 and 2 and 3; P-value‡: comparison between group 3 and 4.
Abbreviations: HBsAg(+), HBV-S antigen positive; HCV-Ab(+), HCV antibody positive; NRTIs, nucleoside reverse-transcriptase inhibitors; NNRTI, non-nucleoside reverse-transcriptase inhibitors; PI, proteinase inhibitor; INSTI, integrase inhibitor; NA, not acquired; eGFRcr, estimating glomerular filtration rate based on creatinine; eGFR$_{Cysc}$, estimating glomerular filtration rate based on cystatin C.

Dynamic of Serum Creatinine and eGFR$_{cr}$ from Baseline to 48 Weeks Among Four Groups

Serum creatinine and calculated eGFR$_{cr}$ levels were compared at baseline, 4-week, 12 week, 24 week, 36 week and 48 week in four groups. Serum creatinine and eGFR$_{cr}$ levels at 4 weeks after therapy were significantly different from the baseline level in DTG-containing groups (group 1, group 2) whether covered up TDF or not, and keep significant difference at 12 weeks, 24 weeks, 36 weeks and 48 weeks (Figure 1A–D). But there was no significant difference occurred at various time-point in control group (Figure 1E and F). Since most of the patients switch-to DTG have experienced long-term treatment, their supervision was not regular as treatment-naïve patients. Even that, the results suggested the significant increased creatinine and decreased eGFR$_{cr}$ at 24 week and 48 week when compared to the baseline (Figure 1G and H). In particular, there were no difference of serum creatinine or eGFR$_{cr}$ level change between time-point of 4w, 12w,24w,36w and 48w in DTG-containing groups.

Cross-Group Comparison of Creatinine and eGFR$_{cr}$ Levels

A cross-group comparison of creatinine and eGFR$_{cr}$ levels at the different treatment timepoints showed a significant difference between the groups. Apart from pre-treatment serum creatinine and eGFR$_{cr}$ level, creatinine was significant higher and eGFR$_{cr}$ levels was significantly lower in group 1 and group 2 when compared with control group (0w: $p=0.99$, $p=0.85$, respectively; 4w,12w,24w, 48w: all of p<0.05; Supplementary Figure 1A and 1C), but no difference between group 1 and group 2 (both p>0.05). We also compared inter-group change of serum creatinine and eGFR$_{cr}$ from baseline till 48 week, there were no difference between group 1 and group 2 but significant increase of serum creatinine and decrease of eGFR$_{cr}$ in comparison with control group (Supplementary Figure 1B and D). As for switch-to DTG-containing group, baseline serum creatinine was slightly higher than control group while eGFR$_{cr}$ level was equal to control group ($p=0.0182,p=0.0506$, respectively, Supplementary Figure 2A and B). At 24 week and 48 week, the significantly higher-level of creatinine and lower eGFR$_{cr}$ were persisted in switch-to DTG-containing group than control group (see in Supplementary Figure 2).

CysC and eGFR$_{CysC}$ Level Dynamics During the 12 Months of Therapy

An analysis of dynamics of CysC levels across the 12th month of therapy showed no significant difference between group 1 and group 2 (Figure 2A and C:$p=0.062$, $p=0.482$ respectively). The eGFR calculated from CysC does not suggest a significant difference among the baseline value, 4w,12w,24w,36w,48 weeks in the above groups (Figure 2B and D; Group 1 and Group 2: $p=0.1936,p=0.1282$, respectively). In particular, no significant change in the CysC and eGFR$_{CysC}$ level were found in the Switch-to DTG group in all time-point (Figure 2G and H; $p=0.4904$, $p=0.09$, respectively). Interestingly, the CysC level has changed significantly in the control group, which initiated HIV treatment with 3TC+TDF+EFV. The CysC decreased from baseline till 4 weeks and 12 weeks ($p=0.281$, $p=0.04$, respectively), while the CysC level in the 4 weeks was significantly different from CysC in 12w, 24w, 36w and 48w (Figure 2E: $p=0.0022$, $p=0.0085$, $p=0.0108$, $p=0.006$, respectively). When compared CysC and eGFR$_{CysC}$ cross-group at different time, we found that mean CysC was lower in control group than TDF+DTG-containing group and DTG-containing group at some follow-up time (Supplementary Figures 3A and C), for instance, significant higher CysC and eGFR$_{CysC}$ in DTG +TDF-containing group and DTG-containing group than control group at 12 week (Supplementary Figure 3A:$p=0.0026$, $p=0.010$; Supplementary Figure 3C:$p=0.0042,p=0.0049$, respectively), which verified the decreased level of Cystatin C in control group. There was no significant difference between DTG+TDF-containing group and DTG-containing group. The median change of CysC and eGFR$_{CysC}$ suggested no difference between three groups (Supplementary Figure 3B and D). Also, CysC dynamics in different time points were described personally (Supplementary Figure 4).

## The Significant eGFR$_{cr}$ Reduction Was Higher in DTG-Treatment Group

Considering >30% eGFR$_{cr}$ decrease than pretreatment or eGFR$_{cr}$ lower than 60 mL/min per 1.73 m2 were meaningful in clinical practice, we divided them into different level of eGFR$_{cr}$ decrease in four groups (Table 2). The results suggested significant higher percentage of patients had decreased eGFR$_{cr}$ in DTG+TDF group and DTG without TDF group when

eGFR from creatine, CKD-EPI
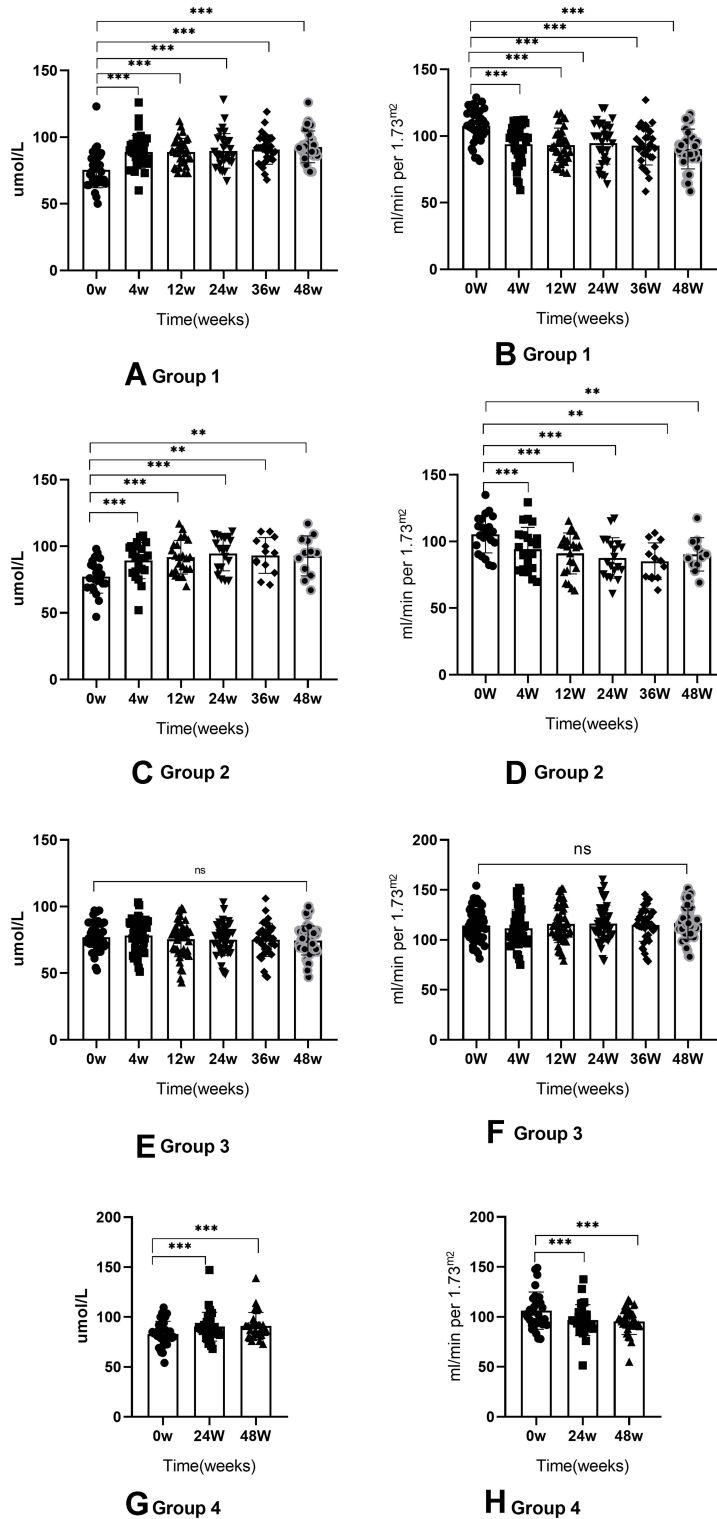
Figure 1 The dynamics of serum creatinine and eGFR$_{cr}$ among four groups (** <0.01,***<0.001; ns: not significant). (**A, C, E** and **G**) showed the mean level of serum creatinine from baseline to 48w. (**B, D, F** and **H**) showed the mean estimating glomerular filtration rate based on serum creatinine, and the equation used CKD-EPI, was expressed as equation depicted above.

eGFR from Cystatin C CKD-EPI
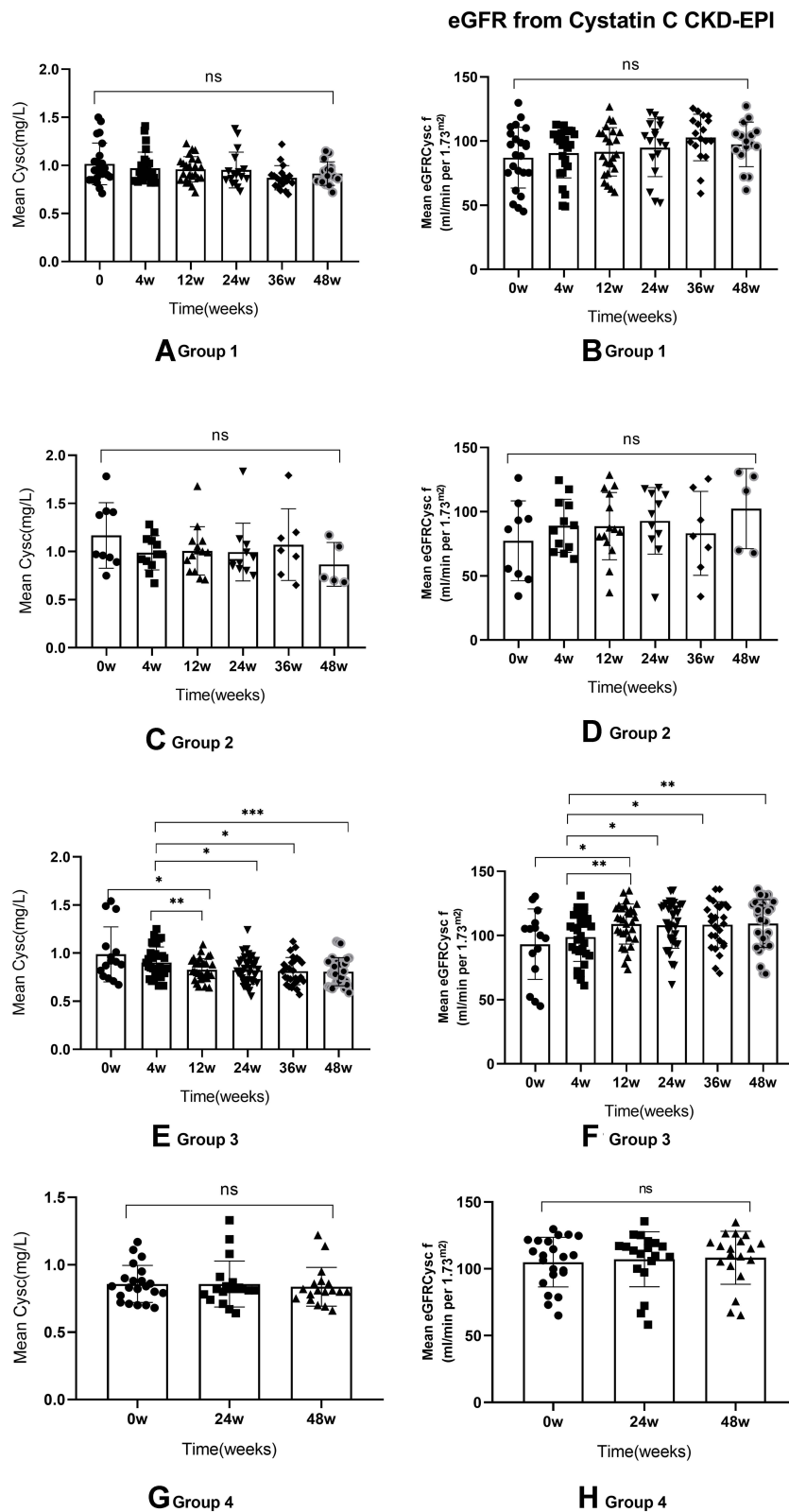
Figure 2 The dynamic of CysC and eGFR$_{CysC}$ among four groups (* <0.05; ** <0.01,***<0.001; ns: not significant). (**A, C, E** and **G**) showed the mean level of cystatin C from baseline to 48w. (**B, D, F** and **H**) showed the mean estimating glomerular filtration rate based on cystatin C, and the equation used CKD-EPI, was expressed as equation depicted above.

Table 2 Decreased Proportion of eGFRcr (or eGFR$_{Cysc}$) When Compared with Baseline Levels Among Four Groups

Decreased Proportion of eGFR	Group 1	Group 2	Group 3	Group 4	p-value[†]	p-value[‡]
>10% from baseline	30(81.1%)	19(82.6%)[a]	20(42.5%)	19(61.3%)	<0.001	0.11
>20% from baseline	23(62.2%)	12(52.2%)[b]	6(12.7%)	9(29.1%)	<0.001	0.07
>30% from baseline	16(43.2%)	3(13.1%)[c]	0 (0.0%)	3(9.6%)	<0.001	0.03
<60(mL/min per 1.73 m2;)	3(8.1%)	1(4.3%)	0(0.0%)	1(3.2%)	0.15	0.40
<60 (mL/min per 1.73 m2; eGFRcysc)	5(13.5%)	5(21.8%)	3(6.3%)	1(3.2%)	0.17	0.92

Notes: P-value[†]: ANOVA test between group 1 and group 2 and group 3; P-value[‡]: comparison between group 3 vs group 4; a, b, c denotes comparison between group 1 and group 2: p=0.881, p=0.445, p=0.014 respectively.
Abbreviations: eGFRcr, estimating glomerular filtration rate based on creatinine; eGFR$_{Cysc}$, estimating glomerular filtration rate based on cystatin C.

compared with the control group. In particular, no significant difference was found between combination DTG with TDF group and DTG without TDF group when their eGFR$_{cr}$ decrement around 10–20% (p=0.881; p=0.445, respectively). However, a significant higher proportion of patients who had decreased eGFR$_{cr}$>30% were found in combination DTG with TDF group when compared with group 2 and group 3 (p=0.014; p<0.001, respectively).

In addition, the number of patients whose eGFR$_{cr}$<60 mL/min per 1.73 m^2 were 3 (8.1%), 1 (4.3%), 0 (0.0%), 1 (3.2%) in group 1, group 2, group 3, group 4 respectively (group 1 vs group 2 vs group 3: p=0.15; group 4 vs group 3: p=0.40, respectively). In group 1, eGFR$_{cr}$<60 mL/min per 1.73 m^2 among three patients occurred at 4w, 36w, 48w who had no history of diabetes or hypertension or co-current nephrotoxic drugs. In group 2, the only one patient had a history of hypertension while the patient in group 4 had no spectacular history but with baseline serum level upper limit of normal.

Individuals with eGFR$_{CysC}$ <60 mL/min per 1.73 m^2 were also identified. Five, five, three, and one subjects from group 1 to group 4, respectively, met these criteria. However, 4/5,4/5,3/3,0/1 subjects had documents for baseline <60 mL/min per 1.73 m^2 instead of significant decrements after treatment. The left one person in group 1 had a history of nephrotoxic drugs and eGFR$_{CysC}$ returned towards the "normal" range over the study period and another patient in group 2 had a history of hypertension. Besides, there were three patients who had obviously abnormal basic urine routine tests (urinary protein>3g) in group 1, group 2 and group 4 when they started ART due to their commodities such as diabetes. However, there were no deterioration of urinary protein and qualitative determination of urinary protein when they were in follow-up. The other patients who have abnormal basic urine routine tests (usually urinary protein 0.3–1g) transiently showed no sign of continuous worsen renal function during ART-treatment.

Measurement of eGFR$_{cr}$ and eGFR$_{CysC}$ Were Less Consistent in DTG-Treatment Patients

Bland–Altman diagram and Intra-class correlation coefficient (ICC) were used to assess consistency of eGFR$_{cr}$ and eGFR$_{CysC}$ from the same sample. Considering relatively small sample size collected in DTG-treatment patients, we gathered patients who used DTG (group 1 and group 2) as a whole when comparing with control group (group 3). The ICC value in DTG-treatment patients was 0.6339 (95% CI: 0.4829 to 0.7408), lower than control group, which value was 0.7588 (95% CI 0.6742 to 0.8215). It usually considers consistency to be better when the ICC value is over 0.75. Bland–Altman diagram showed average bias and variation of "bias" visually (Figure 3A and B). The mean difference was −3.1 mL/min per 1.73 m^2 and 9.8 mL/min per 1.73 m^2 in DTG-treatment group and control group, respectively. The bias was less significant in clinical practice. As can be seen from the figure, 5.34% of the points (7/131) are beyond 95% LoA (− 48.1~ 44.1) in DTG-treatment group (Figure 3A), while 4.06% of the points (7/172) are beyond 95% LoA (− 21.9~ 41.5) in control group (Figure 3B).

Discussion

In our study, two types of biomarkers of kidney function were evaluated in HIV-positive patients who received different ART regimens over a 48-week follow-up period. ART is highly effective in virologic suppression of HIV infection and early onset of ART reduces mortality. Whereas, some antiretroviral drugs, like TDF-associated nephrotoxicity, have
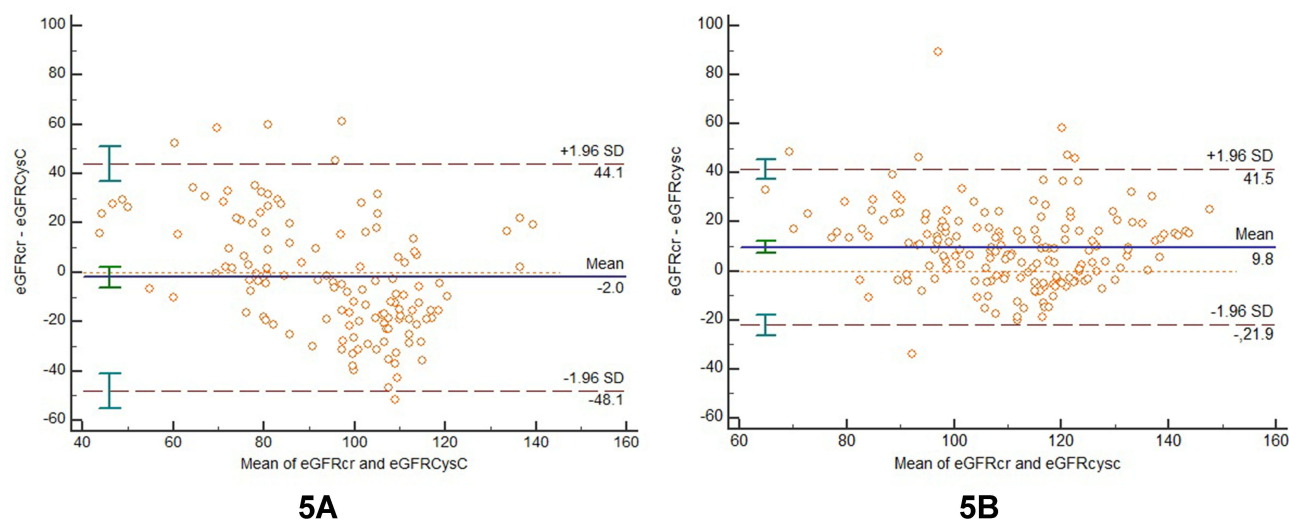
Figure 3 Bland–Altman of consistency between eGFR$_{cr}$ and eGFR$_{CysC}$ among DTG-treatment group (**5A**, group 1 and group 2) and control group (**5B**). The horizontal axe is the mean value of eGFR$_{cr}$ and eGFR$_{CysC}$ results and the vertical axe is the difference value of eGFR$_{cr}$ and eGFR$_{CysC}$. The solid blue lines represent the mean value of the difference. The top and bottom dotted lines are the 95% limits of agreement (95% LoA).

potential to accelerate decline in estimated glomerular filtration rate (eGFR) and cause proximal tubular dysfunction.[5,17] The newly developed integrase inhibitor dolutegravir was reported to elevate serum creatinine mildly regardless of combination with other ART medications or two-drug therapy.[18,19] Therefore, elevated serum creatinine or decreased eGFR$_{cr}$ during treatment should be properly interpreted and treatment should be censured accordingly.

Our results suggested that DTG-containing therapeutic regimens presented higher serum creatinine elevation and decreased eGFR$_{cr}$ than TDF-containing group until 48 weeks, which was consistent with results from randomized clinical trials.[9,18–20] In healthy people, Koteff et al demonstrated that administration of DTG decreased serum creatinine clearance rate(CL$_{cr}$) by 10–14%.[21] Venter et al[18] compared tenofovir alafenamide fumarate/ emtricitabine/dolutegravir (TAF/FTC/DTG) group, TDF/FTC/DTG group and TDF/FTC/EFV found that at 48 week, creatinine clearance rate was significant lower in DTG-containing group (median −16 mL/min) and lower in the TAF-base (median −13mL/min) group than in the TDF-based (median −2.5mL/min) group. Similarly, the combined 48-week results from the DTG randomized clinical trials showed reductions in creatinine clearance DTG (−16.5 mL/min), implying that the effects on renal tubular may be common.[9]

In our results of group 1 and group 2, eGFR$_{cr}$ decreased significantly at 4 week but no significant decline after 4 weeks, which may mainly due to the inhibition of organic cation transporter 2 (OCT2) by DTG instead of tenofovir. We found that TDF co-administered with lamivudine (3TC) and efavirenz (EFV) have shown no effect on serum creatinine and eGFR$_{cr}$ until 48 weeks and the proportion of mild eGFR$_{cr}$ change were minor than DTG-containing therapeutic groups. In consistency, clinical trials have shown initial reductions in eGFR$_{cr}$ of 10–15% in patients in whom TDF combined with cobicistat(COBI)-boosted protease could reach a new steady state after 4 weeks, with no further decline up to 2 year.[22] Whereas TDF [co-administered with emtricitabine (FTC) and efavirenz (EFV)] have shown no effect on serum creatinine and eGFR$_{cr}$ to 48 weeks in ART-naïve patients.[23,24] Besides, higher percentage of severe (>30% eGFR$_{cr}$ decrease or eGFR$_{cr}$ lower than 60 mL/min per 1.73 m2) eGFR$_{cr}$ decrement was observed in DTG+TDF containing group and DTG-containing group than control group. Although we have not observed any actual event of kidney damage in patients, it will interfere with the clinical judgment of "real or fake" renal function change. In this way, we need either seek for reliable but convenient renal biomarkers for clinical judgement or try to build new set-point of "baseline" serum creatinine or eGFR$_{cr}$ level, for example, approximately 4 weeks after initiation of these drugs.

The study in Japanese suggested that no differences in cystatin C and eGFR$_{Cysc}$ between baseline and at 48 weeks after DTG administration and eGFR$_{CysC}$ was more accurate than eGFR$_{cr}$ (40% accuracy compared to 93%).[25] Another study showed with a similar result of +8.6 μmol/L increased serum creatinine values but no significant change in the

cystatin C value variation and associated estimated GFR.[26] These findings were consistent with our study, which did not reveal any changes in longitudinal cystatin C and cystic eGFR within 48 weeks of treatment with DTG. Bland–Altman diagram and Intra-class correlation coefficient of $eGFR_{cr}$ and $eGFR_{CysC}$ proved the less consistent results in DTG-treatment patients rather than TDF-treatment patients. However, we must take into account that the level of cystatin C may have been minimally affected by chronic inflammation caused by HIV infection or higher viral load,[27] as CysC and $eGFR_{CysC}$ in control group had a slight but significant change from 4 week to 48 week in our results which is not consistent with serum creatinine and $eGFR_{cr}$.

In real-world comparison study, Casado et al[28] evaluated kidney function in patients treated by one or two inhibitors of the tubular secretion of creatinine with serum creatinine and cystatin C at 48 week. Another cross-sectional study compared the means of $eGFR_{CysC}$ and $eGFR_{Cr}$ on patients switch to DTG and concluded that cystatin C was probably more relevant from a clinical perspective.[29] Although these studies may have larger sample size and more type of regimens (inhibitors of OCT2), our study compared serum creatinine and Cystatin C in a more comprehensive manner, and calculated eGFR based on them in the transversal and longitudinal treatment of HIV by DTG and/or TDF. In addition, our results may thus be particularly interesting as we were able to compare cystatin C-based formulas with creatinine-based formulas to estimate GFR in patients treated naïve with DTG and switch-to DTG. Finally, we included patients with PI-based ART (LPV/r) before switch-to DTG-based regimen, which may have potential effect on renal function when combined with TDF on the literature data.[30–32] Whether the "overlap" effect would last until switch-to ART regimen remained unknown. Besides, patients with chronic disease before ART treatment, such as uncontrolled diabetes, hypertension, have a higher risk of progression of kidney disease and their kidney function observation were included. For instance, DTG may worsen diabetes management in HIV-infected patients because its chelating effect of mg^{2+} may undermine insulin action.[33,34] Their kidney function should be monitored more closely and in combination with more renal biomarkers to decide on appropriate antiretroviral therapy.

The limitations of this study include this study was single-center, retrospective design and a relatively small sample size, and we could not document the laboratory parameters strictly like randomized clinical trials. Moreover, we did not include more biomarkers for weighing renal function, for example, albumin, Beta-2-microglobulin (β2-MG), Urinary Albumin to Creatinine Ratio, Microalbuminuria/Creatinine, which may be sensitive but less convenient for monitoring. Finally, we could not confirm our results by measuring iohexol clearance, the gold standard method to assess the GFR.

In conclusion, we found that change of serum creatinine level in DTG-containing ART in clinical practice needs for attention due to a part of elevation can be attributed to DTG effect on renal serum creatinine secretion, especially combined with tenofovir or events having potential damage to the renal function. And it is more stable using Cystatin C and $eGFR_{CysC}$ levels for assessing glomerular filtration rate than merely serum creatinine and $eGFR_{cr}$ when evaluating renal function in DTG-treatment patients.

Abbreviations

DTG, dolutegravir; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine; EFV, efavirenz; CysC, cystatin C; FTC, emtricitabine; LPV/r, lopinavir and ritonavir; ART antiretroviral treatment; TAF, tenofovir alafenamide fumarate; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFRcr, estimated glomerular filtration rate based on serum creatinine; eGFR$_{Cysc}$, estimated glomerular filtration rate based on cystatin C; COBI, cobicistat; CL_{cr}, serum creatinine clearance rate; NRTIs, nucleoside reverse-transcriptase inhibitors; NNRTI, non-nucleoside reverse-transcriptase inhibitors; PI, proteinase inhibitor; INSTI, integrase inhibitor. HBsAg(+), HBV-S antigen positive; HCV-Ab(+), HCV antibody positive; β2-MG, Beta-2-microglobulin.

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Disclosure

The authors declare that they have no competing interests.

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