Table 4 Pre- and post-procedural radiographic parameters in the 25 patients with DND who achieved CMI

Radiographic parameters	Pre-PVP	Post-PVP (1 month)	Final follow up (24 months)
Local kyphotic angle (°)	16.4 ± 8.1	6.5 ± 9.3	13.8 ± 13.2
Percent spinal canal compromise (%)	32.4 ± 11.9	31.9 ± 10.5	35.5 ± 9.8
Intravertebral instability (°)[†]	9.6 ± 4.0	2.6 ± 2.2	3.7 ± 2.2

Plus-minus values are means ± 1SD. There were no significant differences in local kyphotic angle and percent spinal canal compromise between pre- and post-PVP (by the Student's *t* test)

DND delayed neurologic deficit, *CM* clinically meaningful improvement, *PVP* percutaneous vertebroplasty

[†] $P < 0.001$ for comparison between pre- and post-PVP

Table 5 Cement leakage outside vertebra

Location	DND group ($n = 30$)	Non-DND group ($n = 214$)	P value[‡]
Epidural vein—no. (%)	0 (0)	3 (1.4)	0.67
Perivertebral soft tissue—no. (%)	0 (0)	11 (5.1)	0.23
Intravertebral disk space—no. (%)	3 (10)	39 (18.2)	0.49
Lung—no. (%)	0 (0)	0 (0)	–
Total—no. (%)	3 (10)	53 (24.8)	0.07

[‡] Between both groups, comparisons for occurrence rates of cement leakage were analysed with Fisher's exact test and Chi-square test

Discussion

This study showed that intravertebral instability and spinal canal compromise could result in the development of DND. In particular, intravertebral instability is considered to be the predominant cause of DND. Percutaneous vertebroplasty is an effective and safe intervention providing clinically meaningful improvement. We have demonstrated that percutaneous vertebroplasty can be readily performed by injection of PMMA into the IVC with the assistance of a cavitygram.

In the literatures, the following factors have been suspected as causes of DND: (1) neural compression secondary to retropulsed bone fragments; (2) progression of kyphosis with vertebral collapse; and (3) intravertebral instability at the fracture site. In accordance with this theory, several types of surgical decompression and fusion through either an anterior and/or posterior approach have been performed [1, 12, 15, 19, 26, 31, 34, 37]. However, some authors have reported that conservative treatment could provide reliable neurologic improvement. Heggeness et al. [13] performed a retrospective study on nine cases of OVCF with IVC with DND. Three with profound and evolving neurologic deficits were treated surgically. Six with significant medical problems and/or relatively minor motor deficits were managed non-operatively. Patients treated surgically recovered full neurologic function, while patients treated non-operatively recovered objective neurologic function. Ataka et al. [2] performed posterior instrumented fusion without neural decompression as treatment for 14 consecutive patients with DND. These procedures provided neurologic improvement and relief of back pain without major complications. They hypothesized that intravertebral instability at the fracture site rather than neural compression is the major cause of DND. In our study, prior to vertebroplasty, we found no significant difference in local kyphotic angle between patients with and without DND. Percent spinal canal compromise and intravertebral instability were greater in the DND group than in the non-DND group. After vertebroplasty, patients with DND who achieved CMI showed substantial improvement of intravertebral instability, although no improvements were attained in local kyphotic angle or percent spinal canal compromise. Our results support the hypothesis that intravertebral instability represents the main cause of DND.

No definitive surgical options are available for the treatment of OVCF with IVC with DND. A combined anterior and posterior procedure may maximize the chances for successful fusion, particularly with multiple points of spinal fixation and occasionally with PMMA augmentation [18]. However, large surgical interventions are still challenging for patients of advanced age, with medical comorbidities, or with poor fixation secondary to osteoporosis [29]. As the population ages, numbers of patients with OVCF with IVC with DND are rapidly increasing. Setting a guideline for the treatment of this pathology is an urgent issue needing to be tackled.

There are some reports about the incidence of cement leakage in vertebroplasty for OVCF with or without IVC. Nieuwenhuijse et al. [23] and Ha et al. [11] reported that there were higher rates of cement leakage in patients with

IVC. On the other hand, Tanigawa et al. [35] reported that there was no statistically significant difference in the incidence of cement leakage between OVCF with cleft and without cleft. Although the differences of the results of these studies are unclear, there is a risk of cement leakage in vertebroplasty for OVCF. In our series, bone needles were inserted into an IVC through a bilateral transpedicular approach and IVC was specifically targeted for PMMA injection. Furthermore, cavitygram of the IVC was performed and measured the capacity of the intravertebral cleft in order to increase the risk of cement leakage. PMMA was injected from the one-sided needle with low pressure and filled the intravertebral cleft. In our series, the rate of incidence of cement leakage was observed in 56 of 244 (23.0 %). This rate of cement leakage seems to be low compared with other studies [11, 23, 35]. PMMA injection into the IVC after cavitygram might reduce the risk of cement leakage.

The current study identified intravertebral instability as the main cause of DND with OVCF with IVC and found that vertebroplasty could be readily performed with injection of the same amount of PMMA as the capacity of the cleft, to stabilize the affected vertebra. Based on our results, we believe that vertebroplasty could be an alternative method for the treatment of OVCF with IVC with DND, as an effective and safe intervention. However, vertebroplasty for patients with severe spinal canal compromise and less intravertebral instability could not achieve any neurologic improvement. Vertebroplasty is not indicated for OVCF with IVC with DND that is affected by static factors other than intravertebral instability. Our study could not statistically describe the cut-off value for spinal canal compromise or intravertebral instability between the CMI group and the non-CMI group because of the small number of patients who failed to achieve CMI. To establish the concrete algorithm for the treatment of this pathology, we should perform a comparative study between vertebroplasty and surgical treatment. However, the significant co-morbidities of our elderly patients with advanced osteoporosis made it difficult to set a randomized controlled trial.

Conclusions

Intravertebral instability and spinal canal compromise could result in the development of DND. In particular, intravertebral instability is considered to be the predominant cause of DND. Percutaneous vertebroplasty is an effective and safe intervention providing clinically meaningful improvement. We have demonstrated that percutaneous vertebroplasty can be readily performed by injection of PMMA into the IVC with the assistance of a cavitygram. It is not suggested that this procedure is applicable to the overall cases of OVCF with IVC with DND. Patients with less intravertebral instability and severe spinal canal compromise should be considered as candidates for spinal reconstructive surgery.

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Conflict of interest None.

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ORIGINAL ARTICLE

Urinary beta-2 microglobulin and alpha-1 microglobulin are useful screening markers for tenofovir-induced kidney tubulopathy in patients with HIV-1 infection: a diagnostic accuracy study

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Abstract Kidney tubulopathy is a well-known adverse event of antiretroviral agent tenofovir. A cross-sectional study was conducted to compare the diagnostic accuracy of five tubular markers, with a collection of abnormalities in these markers as the reference standard. The study subjects were patients with HIV-1 infection on ritonavir-boosted darunavir plus tenofovir/emtricitabine with suppressed viral load. Kidney tubular dysfunction (KTD) was predefined as the presence of at least three abnormalities in the following five parameters: β2-microglobulinuria (β2M), α1-microglobulinuria (α1M), high urinary *N*-acetyl-β-D-glucosaminidase (NAG), fractional excretion of phosphate (FE_{IP}), and fractional excretion of uric acid (FE_{UA}). Receiver operating characteristic curves and areas under the curves (AUC) were estimated, and the differences between the largest AUC and each of the other AUCs were tested using a nonparametric method. The cutoff value of each tubular marker was determined using raw data of 100 % sensitivity with maximal specificity. KTD was diagnosed in 19 of the 190 (10 %) patients. The AUCs (95 % CIs) of each tubular marker were β2M, 0.970 (0.947–0.992); α1M, 0.968 (0.944–0.992); NAG, 0.901 (0.828–0.974); FE_{IP}, 0.757 (0.607–0.907), and FE_{UA}, 0.762 (0.653–0.872). The AUCs of β2M and α1M were not significantly different, whereas those of the other three markers were smaller. The optimal cutoff values with 100 % sensitivity were 1,123 μg/gCr (β2M, specificity 89 %), 15.4 mg/gCr (α1M, specificity 87 %), 3.58 U/gCr (NAG, specificity 46 %), 1.02 % (FE_{IP}, specificity 0 %), and 3.92 % (FE_{UA}, specificity 12 %). Urinary β2M and α1M are potentially suitable screening tools for tenofovir-induced KTD. Monitoring either urinary β2M or α1M should be useful in early detection of tenofovir nephrotoxicity.

Keywords Tenofovir · Kidney tubular dysfunction · Screening · Urinary beta-2 microglobulin · Urinary alpha-1 microglobulin · HIV-1 infection

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Introduction

Tenofovir disoproxil fumarate (TDF), a prodrug of tenofovir, is a widely used nucleotide reverse transcriptase inhibitor (NRTI) as part of the initial antiretroviral therapy for patients with human immunodeficiency (HIV) infection, as well as in treatment of hepatitis B virus infection [1–4]. Tenofovir is excreted through the kidney by glomerular filtration and active tubular secretion. Renal proximal tubular damage is a well-known adverse effect of tenofovir, which sometimes leads to diabetes insipidus, Fanconi syndrome, and acute renal failure [5–7], and patients on TDF are more likely to develop renal dysfunction than those on other NRTIs [8, 9]. Of note, low

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bone mineral density, another adverse effect of TDF, sometimes occurs as a result of kidney proximal tubulopathy associated with phosphate wasting and increased bone turnover [10, 11].

The risk of clinically relevant tenofovir-induced nephrotoxicity is considered to be relatively low [9, 12, 13]. However, tenofovir renal safety has not been confirmed in the long term. Importantly, both HIV infection and hepatitis B infection require long-term treatment, especially for HIV infection, where the treatment is lifelong. In tenofovir-induced nephrotoxicity, tubular dysfunction usually precedes the decline in glomerular filtration rate (GFR), suggesting that tubular markers are more sensitive than estimated GFR (eGFR) calculated with serum creatinine in screening for tenofovir nephrotoxicity [9, 14–16]. Thus, it is important to identify a renal tubular marker with high sensitivity to screen for tenofovir-induced KTD.

There is no gold standard for the diagnosis of tenofovir-induced kidney tubular dysfunction (KTD). Previous reports usually applied a collection of abnormalities in tubular function parameters as diagnostic criteria [17, 18]. However, the criteria used in each study differ, and it is often cumbersome and costly to monitor multiple renal tubular markers in practice. To our knowledge, there are no studies that have compared the diagnostic accuracy of various tubular markers for tenofovir-induced KTD. Furthermore, several pathological conditions are associated with KTD, such as active infection, diabetic nephropathy, and preexisting renal impairment [19, 20], making it difficult to evaluate KTD induced exclusively by tenofovir.

Based on the foregoing background, we designed the present study to identify the best screening marker(s) of tubular dysfunction in tenofovir-induced KTD, using a collection of abnormalities in kidney tubular markers as the reference standard.

Patients and methods

Ethics statement

This study was approved by the Human Research Ethics Committee of the National Center for Global Health and Medicine, Tokyo, Japan. Each patient in this study provided a written informed consent for publication of clinical data for research purpose. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study design

This study was designed and reported according to the recommendations of the standards for reporting of diagnostic accuracy (STARD) statement [21]. We performed a single-center cross-sectional study to compare the diagnostic accuracies of various kidney tubular markers for tenofovir-induced KTD, with a collection of abnormalities in these markers as the reference standard, to identify the best screening marker in tenofovir-treated Japanese patients with HIV infection.

Study subjects

The study population has been previously reported [22]. The study enrolled consecutive Japanese patients with HIV infection, aged >17 years, with HIV-1 viral load <200 copies/ml, and on at least 4-week treatment with once-daily ritonavir (100 mg)-boosted darunavir (800 mg) plus fixed-dose tenofovir (300 mg)/emtricitabine (200 mg), seen at our clinic between October 1, 2011 and March 31, 2012. The exclusion criteria were (1) active infection, (2) malignancy, (3) diabetes mellitus, defined by the use of glucose-lowering agents or fasting plasma glucose >126 mg/dl or plasma glucose >200 mg/dl on two different days, (4) alanine aminotransferase 2.5 times more than the upper limit of normal, (5) estimated glomerular filtration rate (eGFR) calculated by Cockcroft–Gault equation of <50 ml/min [creatinine clearance = [(140 − age) × weight (kg)]/(serum creatinine × 72)(×0.85 for females)] [23], and (6) patients who did not sign the consent form.

Measurements

Blood and spot urine samples were collected on the same day (either on the day of enrollment or on the next visit), together with body weight measurement. The blood samples were used to measure serum creatinine, serum uric acid, serum phosphate, CD4 count, and C-reactive protein (CRP); urine samples were used to measure phosphate, uric acid, creatinine, β2-microglobulin (β2M), α1-microglobulin (α1M), and *N*-acetyl-β-D-glucosaminidase (NAG). The values of β2M, α1M, and NAG measured in the urine samples were expressed relative to urinary creatinine of 1 gl/l (/g Cr) [24].

Urinary concentrations of β2M and α1M were measured with a latex aggregation assay kit (β2M, BMG-Latex X1 "Seiken"; Denka Seiken, Niigata, Japan; α1M, Eiken α1M-III; Eiken Chemical, Tokyo, Japan), and those of NAG by colorimetric assay of enzyme activity with 6-methyl-2-pyridyl-*N*-acetyl-1-thio-β-D-glucosaminide as substrate (Nittobo Medical, Tokyo, Japan).

Definition of renal proximal tubular dysfunction

KTD was predefined as the presence of at least three abnormalities in the following five parameters: fractional excretion of phosphate (FE_{IP}) {[(urine phosphate × serum creatinine)/(serum phosphate × urine creatinine)] × 100} > 18 %,

fractional excretion of uric acid (FE_{UA}) {[(urine uric acid × serum creatinine)/(serum uric acid × urine creatinine)] × 100)} > 15 %, β2-microglobulinuria (β2M > 1,000 μg/g Cr), α1-microglobulinuria (α1M > 16.6 mg/g Cr), and high NAG level in urine (NAG > 5.93 U/g Cr). The definition of KTD and the foregoing cutoff levels were determined based on the published reports [18, 25, 26].

The potential risk factors for KTD were determined according to previous studies and collected together with the basic demographics from the medical records [14, 27–30]: included were age, sex, body weight, and presence or absence of other medical conditions (concurrent use of other nephrotoxic drugs such as ganciclovir, sulfamethoxazole/trimethoprim, and nonsteroidal antiinflammatory agents; coinfection with hepatitis B, defined by positive hepatitis B surface antigen; coinfection with hepatitis C, defined by positive HCV viral load; hypertension, defined by current treatment with antihypertensive agents or two successive measurements of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg in the clinic; dyslipidemia, defined by current treatment with lipid-lowering agents or two successive measurements of either low density lipoprotein cholesterol >140 mg/dl, high density lipoprotein cholesterol <40 mg/dl, total cholesterol >240 mg/dl, triglyceride >500 mg/dl). At our clinic, blood pressure and body weight are measured every visit. We used the data on or closest to and preceding the day of blood/urine sample collection by no more than 180 days.

Statistical analysis

The baseline characteristics of patients with KTD and those without tubular dysfunction were compared by the Student's *t* test for continuous variables (e.g., kidney tubular markers), and by the χ^2 test or Fisher's exact test for categorical variables. Box plots were constructed for tubular markers of KTD and non-KTD. Diagnostic test comparison was performed using KTD as the dichotomous variable. Receiver operating characteristic (ROC) curves were constructed for individual markers, and the area under the curve (AUC) was estimated with 95 % confidence interval. The differences between the largest AUC and each of the other AUCs were tested using a nonparametric method [31], and multiple comparisons were adjusted with the Bonferroni correction. The cutoff value for each tubular marker was determined using raw data of 100 % sensitivity with maximal specificity because this point would diagnose all KTD cases with minimal false positives. For reference, two methods commonly applied for the identification of optimal cutoff points using the ROC curve were also applied: method 1 [the point on the curve closest to the point with sensitivity of one and one minus specificity of zero, calculated as the minimal value for $(1-\text{sensitivity})^2 + (1-\text{specificity})^2$]; and method 2 [the maximum vertical distance between the ROC curve and the diagonal line, calculated as the maximum value for (sensitivity + specificity − 1)] [32]. A p value <0.05 was considered statistically significant. Nonparametric methods to compare AUC of tubular makers were performed with Stata software SE ver. 12 (College Station, TX, USA). All other statistical analyses were performed with the Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL, USA).

Results

A total of 190 patients were enrolled from whom blood and urine samples available for analysis (Fig. 1). Among them, 19 patients (10 %) fulfilled the criteria for KTD. The baseline characteristics and laboratory data for patients

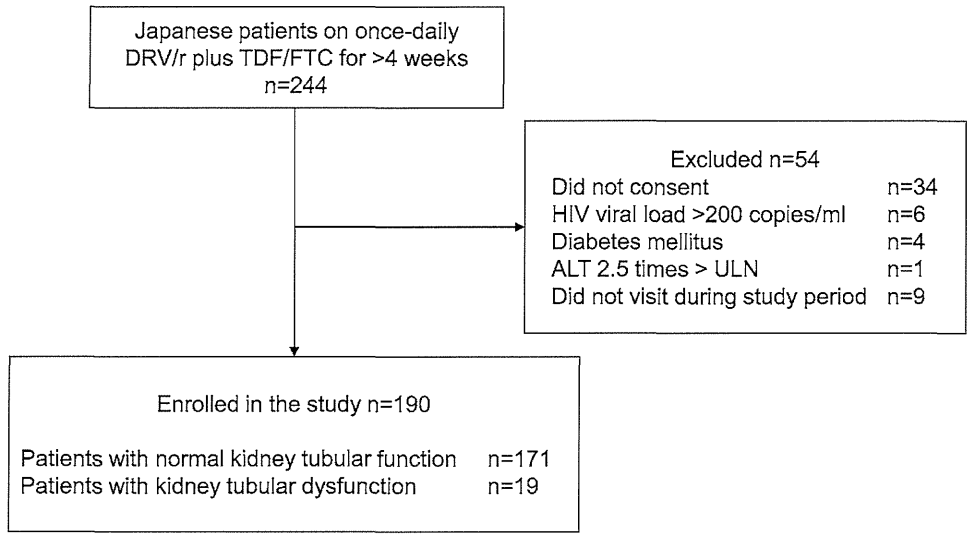


Fig. 1 Patient enrollment. *DRV/r* ritonavir-boosted darunavir; *TDF/FTC* tenofovir/emtricitabine; *ALT* alanine transaminase; *ULN* upper limit of normal

with KTD and patients without tubular dysfunction are listed in Table 1. Patients with KTD were older ($p < 0.001$), had a lower body weight ($p = 0.006$) and lower eGFR ($p = 0.003$), and were more likely to be hypertensive than patients with normal tubular function, although the difference was not significant ($p = 0.088$). The median duration of tenofovir therapy was 71.5 weeks [interquartile range (IQR), 36.8–109.2 weeks] for the entire study population and was not different between the two groups ($p = 0.888$).

Differences in tubular markers between patients with KTD and those without KTD are shown in Table 1 and box-and-whisker plots in Fig. 2. Patients with KTD had higher levels of all five tubular markers with p value <0.001 (Fig. 2). The performance of each tubular marker in differentiating patients with KTD from those with normal tubular function is illustrated by ROC curves (Fig. 3a). The AUCs and 95 % confidence intervals for the diagnosis of KTD by each tubular marker were β2M, 0.970 (0.947–0.992); α1M, 0.968 (0.944–0.992); NAG, 0.901 (0.828–0.974); FE_{IP}, 0.757 (0.607–0.907); and FE_{UA}, 0.762 (0.653–0.872). Results of comparisons of AUCs of β2M (with the largest AUC) and other markers are shown in Fig. 3b. The AUCs of β2M and α1M were not significantly different, whereas the AUCs of both FE_{IP} and FE_{UA} were significantly smaller than that of β2M. The AUC of NAG was marginally smaller than that of β2M with a single test, but the difference was no longer significant after adjustment of Bonferroni correction (Fig. 3b). Thus, urinary β2M and α1M had the best diagnostic performances for detecting KTD.

Identifying optimal cutoff point for tubular markers

The cutoff values for the different tubular markers with 100 % sensitivity and the maximal specificity were as follows: β2M, 1,123.2 μg/g Cr (specificity, 89 %); α1M, 15.4 mg/g Cr (specificity, 87 %); NAG, 3.58 U/g Cr (specificity, 46 %); FE_{IP}, 1.02 % (specificity, 0 %); and FE_{UA}, 3.92 % (specificity, 12 %) (Table 2). The cutoff values determined by the aforementioned two conventional methods are also listed in Table 2. The cutoff values of both β2M and α1M by method 1 yielded the high diagnostic accuracy (β2M, 1,612 μg/g Cr, sensitivity 95 %, specificity 93 %; α1M, 16.5 mg/g Cr, sensitivity 95 %, specificity 90 %), whereas the cutoff values for these two markers calculated with method 2 were the same as the aforementioned ones with 100 % sensitivity and maximal specificity. Methods 1 and 2 yielded the same cutoff value for NAG of 5.96 U/g Cr (sensitivity 90 %, specificity 86 %). For FE_{IP} and FE_{UA}, the sensitivity was relatively low with the cutoffs gained with method 1 and method 2, suggesting that FE_{IP} and FE_{UA} are not useful for screening KTD (Table 2).

Table 1 Characteristics of patients with and without kidney tubular dysfunction (KTD)

	KTD ($n = 19$)	Non-KTD ($n = 171$)	p value
Kidney tubular markers			
β2M > 1,000 μg/g Cr, *n* (%)	19 (100)	21 (12.3)	<0.001
α1M > 16.6 mg/g Cr, *n* (%)	18 (94.7)	17 (9.9)	<0.001
NAG > 5.93 U/g Cr, *n* (%)	17 (89.5)	23 (13.5)	<0.001
Fractional excretion of phosphate >18 %, *n* (%)	5 (26.3)	2 (1.2)	<0.001
Fractional excretion of uric acid >15 %, *n* (%)	2 (10.5)	4 (2.3)	0.112
Characteristics			
Sex (male), *n* (%)	18 (94.7)	166 (97.1)	0.473
Age (years)[a]	60 (41–62)	38 (32–42)	<0.001
Route of transmission (homosexual contact), *n* (%)	16 (84.2)	153 (89.5)	0.528
Weight (kg)[a]	56 (53.5–66.5)	67.2 (58.1–75)	0.006
eGFR (ml/min/1.73 m^2)[a]	75.5 (62.8–93.5)	87.7 (77.5–98)	0.003
Serum creatinine (mg/dl)[a]	0.85 (0.68–0.96)	0.80 (0.73–0.88)	0.168
CD4 cell count (/μl)[a]	380 (194–501)	379 (275–533)	0.261
Serum phosphate (mg/dl)[a]	3.4 (2.7–3.7)	3.2 (2.9–3.6)	0.815
Serum uric acid (mg/dl)[a]	4.7 (4.2–5.7)	5.6 (4.8–6.4)	0.080
Nephrotoxic drugs, *n* (%)	2 (10.5)	12 (7.0)	0.420
Hepatitis C, *n* (%)	0 (0)	3 (1.8)	0.728
Hepatitis B, *n* (%)	2 (10.5)	24 (14)	0.501
Dyslipidemia, *n* (%)	4 (21.1)	54 (31.6)	0.253
Hypertension, *n* (%)	8 (42.1)	42 (24.6)	0.088
C-reactive protein (mg/dl)[a]	0.07 (0.03–0.28)	0.07 (0.03–0.16)	0.277
TDF, weeks[a]	60.3 (17.7–115.4)	73.3 (37.7–109.1)	0.888

KTD kidney tubular dysfunction, *β2M* urinary β2-microglobulin, *α1M* urinary α1-microglobulin, *NAG* N-acetyl-β-D-glucosaminidase in urine, FE_{IP} fractional excretion of phosphate, FE_{UA} fractional excretion of uric acid, *eGFR* estimated glomerular filtration rate, *TDF* tenofovir disoproxil fumarate

[a] Median (interquartile range)

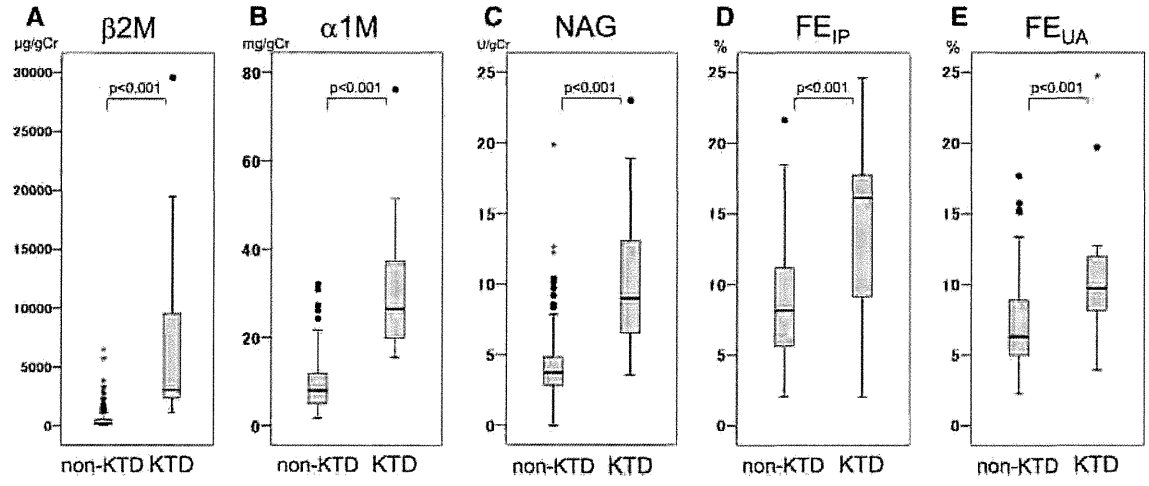
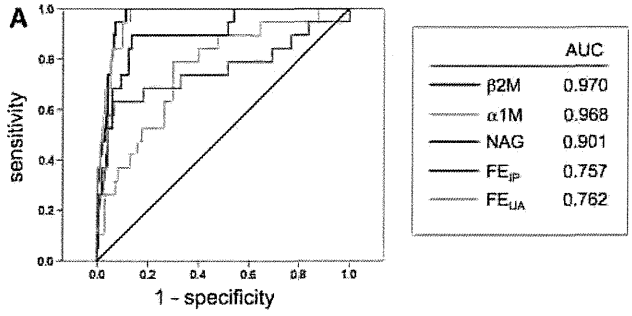


Fig. 2 Box-and-whisker plots of five tubular markers: urinary β2-microglobulin (*β2M*) (**a**), urinary α1-microglobulin (*α1M*) (**b**), *N*-acetyl-β-D-glucosaminidase in urine (*NAG*) (**c**), fractional excretion of phosphate (FE_{IP}) (**d**), and fractional excretion of uric acid (FE_{UA}) levels in patients with kidney tubular function (*KTD*) and those with normal tubular function (*non-KTD*) (**e**). In these plots, *lines within the boxes* represent median values; the *upper* and *lower lines of the boxes* represent the 25th and 75th percentiles, respectively; and the *upper and lower bars outside the boxes* represent the maximum and the minimum values or to the most extreme values within 1.5 interquartile ranges of the quartiles, respectively. *Closed circles* and *asterisks* in each graph represent outliers



B

	AUC	95% CI	Std. Err.	P value	P value†
β2M (standard)	0.970	0.947-0.992	0.011		
α1M	0.968	0.944-0.992	0.012	0.886	1
NAG	0.901	0.828-0.974	0.038	0.093	0.371
FE_{IP}	0.757	0.607-0.907	0.078	0.004	0.018
FE_{UA}	0.762	0.653-0.872	0.057	<0.001	<0.001

Fig. 3 The diagnostic accuracy of five tubular markers for tenofovir-induced tubulopathy. **a** Receiver operating characteristic (ROC) curves and areas under the curve (AUC) for five tubular markers. **b** The differences between the largest AUC (β2M) and each of the other AUCs were tested using a nonparametric method. *p* value†, value adjusted with Bonferroni correction

Discussion

To our knowledge, this is the first study to compare various kidney tubular markers for screening tenofovir-induced KTD in patients with HIV-1 infection. Both urinary β2M and α1M were identified as good screening markers with high diagnostic accuracy among the five tubular markers examined in this study. With a cutoff value of 1,123 μg/g Cr for β2M (sensitivity 100 %, specificity 89 %) and 15.4 mg/g Cr for α1M (sensitivity 100 %, specificity 87 %), these two markers are potentially suited for screening tenofovir-induced KTD. Although these low molecular weight proteins offered good screening ability, both FE_{IP} and FE_{UA}, which are traditional tubular function markers often used for the diagnosis of Fanconi syndrome, were not useful for screening KTD. NAG, a lysosomal enzyme of proximal tubular epithelial cells, had good diagnostic accuracy with a cutoff value of 5.96 U/g Cr (sensitivity 90 %, specificity 86 %). However, with cutoff of 3.58 U/g Cr, which yields 100 % sensitivity and maximal specificity, NAG had relatively low specificity of 46 %, and thus has a high false-positive rate.

TDF is one of the most important and widely used agents in the treatment of HIV-1 infection, as well as hepatitis B infection [4]. Fixed-dose tenofovir/emtricitabine is the only preferred NRTI in the American Department of Health and Human Services (DHHS) Guidelines and the revised British HIV Association Guidelines [33, 34]. TDF is also increasingly used in resource-limited settings, following the revised 2010 WHO guidelines that recommend TDF as one of the components of first-line therapies [35]. Although tenofovir nephrotoxicity is considered to be mild and tolerable [9], its long-term consequences are unknown. Thus, it is important to have a useful screening tool for tenofovir-induced nephrotoxicity.

Table 2 Cutoff values of five kidney tubular markers (calculated with 100 % sensitivity and maximal specificity) and two conventional methods

	Cutoff with 100 % sensitivity			Method 1			Method 2		
	Cutoff	Sensitivity (%)	Specificity (%)	Cutoff	Sensitivity (%)	Specificity (%)	Cutoff	Sensitivity (%)	Specificity (%)
β2M (μg/g Cr)	1,123	100	89	1,612	95	93	1,123	100	89
α1M (mg/g Cr)	15.4	100	87	16.5	95	90	15.4	100	87
NAG (U/g Cr)	3.58	100	46	5.96	90	86	5.96	90	86
FE_{IP} (%)	1.02	100	0	12.4	68	82	14.4	63	94
FE_{UA} (%)	3.92	100	12	8.1	79	70	8.1	79	70

β2M urinary β2-microglobulin, *α1M* urinary α1-microglobulin, *NAG* N-acetyl-β-D-glucosaminidase in urine, FE_{IP} fractional excretion of phosphate, FE_{UA} fractional excretion of uric acid

Previous studies identified old age, low body weight, preexisting renal impairment, concomitant use of nephrotoxic medications, concomitant use of ritonavir-boosted protease inhibitors, advanced HIV infection (low CD4 counts, AIDS), and other comorbidities (diabetes mellitus, hypertension, hepatitis C co-infection) as risk factors for tenofovir-induced reduction in renal function [14, 27–30]. The DHHS Guidelines recommend monitoring eGFR, urinalysis, and electrolytes in patients on TDF [33]. We suggest monitoring either urinary β2M or α1M in addition to the variables recommended by the DHHS guidelines every 6 months in patients under tenofovir use, especially in those with the aforementioned risk factors in resource-rich settings.

One of the strengths of the present study is the exclusion of factors that could otherwise predispose to KTD other than tenofovir, such as active infection, diabetes mellitus, preexisting renal impairment, and HIV-1 viremia, to make prevalence of KTD lower than that in the real-world settings [20]. The cutoff values of tubular markers for screening tenofovir-induced KTD with 100 % sensitivity were calculated in this setting. Thus, in applying these cutoff values in clinical practice with high prevalence rates of KTD, the false-positive rate will be lower than the one reported in the present study, making these cutoff values even more useful.

Another strength of the study is that the enrolled patients were on the same antiretroviral regimen (once-daily ritonavir-boosted darunavir plus fixed-dose tenofovir/emtricitabine). This practice helped proper evaluation of the diagnostic accuracy of the five tubular markers, because plasma concentrations of tenofovir and severity of tenofovir-induced KTD are influenced by concomitant use of antiretrovirals, and the delta change in plasma tenofovir concentration likely differs in the presence of each concomitant drug [36]. Thus, by enrolling patients on the same antiretroviral combination, this study excluded an important confounding factor for tenofovir-induced KTD.

Both β2M and α1M are low molecular weight proteins (<40 kDa) used as markers of kidney tubular function. β2M and the free unbound form of α1M are freely filtered by the glomerulus and reabsorbed almost completely in proximal tubular cells [37]. Serum β2M has been used as a surrogate marker of inflammation, because it is expressed on the surface of most nucleated cells, as part of class I major histocompatibility complex. On the other hand, α1M is mainly produced in the liver, and although its function is not fully understood, it has antioxidant properties and acts as a radical scavenger [38]. Of note, technical difficulty has been reported in the measurement of both markers; for β2M, acidic urine with pH <6.0 causes time- and temperature-dependent degradation of β2M [39]. Urinary α1M is more stable than β2M when stored in acidic urine; however, diurnal variation and gender differences have been reported [40–42].

There are several limitations in this study. First, there is no gold standard definition for KTD. The collection of abnormal tubular markers was used as a reference standard in this study, following their use in previous studies for the diagnosis of KTD [17, 43]. However, the criteria used for the diagnosis of KTD in each previous study included haphazard combination of tubular markers [16, 44]. Accordingly, this study selected five markers (β2M, α1M, NAG, FE_{IP}, and FE_{UA}) after taking into consideration their availability and cost. Thus, our study did not investigate other tubular markers, such as γ-glutamyl transpeptidase, retinol binding protein, and neutrophil gelatinase-associated lipocalin. Second, although this study evaluated the diagnostic values for five variables (β2M, α1M, NAG, FE_{IP}, and FE_{UA}), these variables were not fully independent from KTD, the reference standard, because KTD was defined as the collection of abnormal tubular markers [17, 43]. Third, the study subjects were mostly men, and thus the results of this study are not necessarily applicable to women, especially considering that gender variation should be taken into account in evaluation of α1M [40].

In conclusion, the present study identified urinary β2M and α1M as highly useful screening markers for tenofovir-induced KTD, in a setting designed to exclusively evaluate tenofovir-induced KTD. In the assessment of renal function

in patients under tenofovir therapy, regular monitoring of either urinary β2M or α1M, in addition to urinalysis, serum creatinine, and electrolytes, should be helpful in the diagnosis of early-stage tenofovir-induced KTD. Screening for tenofovir-induced KTD is especially important in patients with several risk factors for KTD, because undetected long-term tubular dysfunction might lead to premature osteopenia caused by phosphate wasting, and accelerated progression of renal dysfunction, both of which could result in a serious outcome.

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Renal Function Declines More in Tenofovir- than Abacavir-Based Antiretroviral Therapy in Low-Body Weight Treatment-Naïve Patients with HIV Infection

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Abstract

Objective: To compare the rate of decline of renal function in tenofovir- and abacavir-based antiretroviral therapy (ART) in low-body weight treatment-naïve patients with HIV infection.

Design: We conducted a single-center retrospective cohort study of 503 Japanese patients who commenced on either tenofovir- or abacavir-based initial ART.

Methods: The incidence of renal dysfunction, defined as more than 25% fall in estimated glomerular filtration rate (eGFR) from the baseline, was determined in each group. The effect of tenofovir on renal dysfunction was estimated by univariate and multivariate Cox hazards models as the primary exposure. Changes in eGFR until 96 weeks were estimated in both groups with a repeated measures mixed model.

Results: The median body weight of the cohort was 64 kg. The estimated incidence of renal dysfunction in the tenofovir and the abacavir arm was 9.84 per 100 and 4.55 per 100 person-years, respectively. Tenofovir was significantly associated with renal dysfunction by univariate and multivariate analysis (HR = 1.747; 95% CI, 1.152–2.648; p = 0.009) (adjusted HR = 2.080; 95% CI, 1.339–3.232; p<0.001). In subgroup analysis of the patients stratified by intertertile baseline body weight, the effect of tenofovir on renal dysfunction was more evident in patients with lower baseline body weight by multivariate analysis (≤60 kg: adjusted HR = 2.771; 95%CI, 1.494–5.139; p = 0.001) (61–68 kg: adjusted HR = 1.908; 95%CI, 0.764–4.768; p = 0.167) (>68 kg: adjusted HR = 0.997; 95%CI, 0.318–3.121; p = 0.995). The fall in eGFR was significantly greater in the tenofovir arm than the abacavir arm after starting ART (p = 0.003).

Conclusion: The incidence of renal dysfunction in low body weight patients treated with tenofovir was twice as high as those treated with abacavir. Close monitoring of renal function is recommended for patients with small body weight especially those with baseline body weight <60 kg treated with tenofovir.

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Introduction

Tenofovir disoproxil fumarate (TDF) and abacavir sulfate (ABC) are widely used nucleot(s)ide reverse transcriptase inhibitors (NRTIs) as part of the initial antiretroviral therapy for patients with HIV infection in the developed countries (URL:http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf) (URL: http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf). TDF is generally preferred to ABC, since ABC is reported to cause serious hypersensitivity reaction in 5–8% of the patients and its efficacy in viral suppression is reported to be inferior to TDF among patients with baseline HIV viral load of >100,000 copies/ml [1,2]. On the other hand, renal proximal tubular damage and renal dysfunction are well-known adverse effects of TDF [3–9]. A meta-analysis study that compared TDF and other NRTIs concluded that the decline in renal function with TDF use is significant but modest, and the ASSERT study conducted in Europe compared randomly-selected treatment naïve patients who commenced treatment with either TDF or ABC with efavirenz and showed no difference in estimated glomerular filtration

rate (eGFR) between the two groups at 48 weeks [9,10]. To date, the nephrotoxicity of TDF have been regarded as mild and tolerable [2,5–7,9–11].

However, the TDF-related nephrotoxicity has hardly been evaluated in patients with small body weight, who are potentially at higher risk for larger drug exposure and thus, more severe toxicity [12–15]. Indeed, some recent studies including ours reported a higher incidence of TDF-related renal dysfunction among Asian patients with low body weight compared with previous studies on mostly Whites and African Americans with larger body weight [13,16]. Thus, it is important to provide more evidence in support of TDF-associated nephrotoxicity in patients with low body weight since such data can elucidate whether TDF-related nephrotoxicity is as mild in low-body-weighted patients as previously reported in Europe and the USA. This is also important because there is increasing use of TDF in resource-limited settings, where patients are often of relatively small body weight, following the revised 2010 WHO guidelines that recommend TDF as one of the components of first line therapies (URL:http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf)[13,16–19]. To our knowledge, there are no studies that compared renal function in treatment naïve Asian patients who commenced treatment with TDF or ABC.

Based on the above background, the present study was designed to compare the incidence of renal dysfunction and change in eGFR between treatment-naïve Japanese patients with low body weight who started either TDF or ABC as part of the antiretroviral regimen.

Methods

Ethics Statement

This study was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine, Tokyo. All patients included in this study provided a written informed consent for their clinical and laboratory data to be used and published for research purposes. This study has been conducted according to the principles expressed in the Declaration of Helsinki.

Study Subjects

We performed a retrospective, single-center cohort study of HIV-infected Japanese patients using the medical records at the National Center for Global Health and Medicine, Tokyo, Japan. Our facility is one of the largest clinics for patients with HIV infection in Japan with more than 2,700 registered patients. The study population was treatment-naïve patients with HIV infection, aged >17 years, who commenced treatment with either the recommended 300 mg/day dose of TDF or 600 mg/day dose of ABC-containing antiretroviral regimen at our clinic between January 1, 2004 and March 31, 2009. During this inclusion period, all except two patients at our clinic started ART with either ABC or TDF. Patients with an eGFR of >60 ml/min/1.73 m^2 were enrolled. Patients were followed up until March 31, 2011. They were excluded if they started ART with both TDF and ABC, their follow-up period at our facility was less than 24 weeks after commencement of ART, or if they had started ART at other facilities. Only Japanese patients were included in order to examine a population with comparatively homogenous basic demographics and background. The attending physician selected either TDF or ABC at baseline, and the use of these two drugs was based on the Japanese guidelines, which place both ABC and TDF as the preferred NRTIs (http://www.haart-support.jp/guideline2011.pdf. in Japanese). The attending physician also selected the key drug [non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), or integrase inhibitor (INI)]. All patients received standard ART with two NRTIs combined with either PI, NNRTI, or INI.

Measurements

We defined renal dysfunction as more than 25% decrease in eGFR relative to the baseline [13,16,20,21]. The baseline eGFR was estimated for each patient from the average of two successive serum creatinine measurements made closest to and preceding the commencement of antiretroviral therapy by no more than 90 days. Changes in eGFR were plotted from the baseline measurement until the average value of two successive measurements diminished to less than 75% of the baseline, discontinuation of TDF or ABC, or at the end of the follow-up period. Discontinuation of TDF and ABC was the choice of the attending physician, and was based on virologic failure or ART-related side effects other than renal dysfunction. Before the initiation of ART and until suppression of HIV-1 viral load, patients visited our clinic every month. However, after viral load suppression, the visit interval was extended up to every three months. Serum creatinine and eGFR were measured in every visit, and the frequency of measurements was similar in patients on TDF and ABC. eGFR was calculated using the equation from the 4-variable Modification of Diet in Renal Disease (MDRD) study, $eGFR = 186 \times [\text{serum creatinine}]^{-1.154} \times [\text{age}]^{-0.203} \times [0.742 \text{ if patient is female}] \times [1.212 \text{ if patient is African American}]$ [22]. In this study, the primary exposure variable was TDF use over ABC as part of the initial ART.

The potential risk factors for renal dysfunction were determined according to previous studies and collected together with the basic demographics from the medical records [15,23–25]. They included age, sex, body weight, body mass index, (BMI) = {body weight (kg) / [(height (m)]^2}, baseline laboratory data (CD4 cell count, HIV viral load, and serum creatinine), and presence or absence of other medical conditions (concurrent use of ritonavir-boosted protease inhibitors, concurrent nephrotoxic drugs such as ganciclovir, sulfamethoxazole/trimethoprim, and non-steroidal anti-inflammatory agents, diabetes mellitus defined by using anti-diabetic agents or fasting plasma glucose >126 mg/dl or plasma glucose >200 mg/dl on two different days, co-infection with hepatitis B defined by positive hepatitis B surface antigen, co-infection with hepatitis C defined by positive HCV viral load, hypertension defined by current treatment with antihypertensive agents or two successive measurements of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg at the clinic, dyslipidemia defined by current treatment with lipid-lowering agents, and current smoking). At our clinic, weight and blood pressure were measured on every visit whereas other variables were measured in the first visit and at least once annually. We used the data on or closest to and preceding the day of starting ART by no more than 90 days.

Statistical analysis

The time to 25% decline in eGFR from the baseline was calculated from the date of commencement of treatment to the date of diagnosis of the above-defined renal dysfunction. Censored cases represented those who discontinued ABC or TDF, dropped out, were referred to other facilities, or at the end of follow-up period. The time from the start of ART to >25% decrease in eGFR was analyzed by the Kaplan Meier method for patients who started TDF (TDF arm) and ABC (ABC arm), and the log-rank test was used to determine the statistical significance. The Cox proportional hazards regression analysis was used to estimate the

impact of TDF use over ABC on the incidence of more than 25% decrease in eGFR relative to the baseline. The impact of each basic demographics, baseline laboratory data, and other medical conditions listed above was also estimated with univariate Cox proportional hazards regression.

To estimate the unbiased prognostic impact of TDF use over ABC for renal dysfunction, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for TDF use over ABC. Model 2 included age and weight plus model 1 in order to adjust for basic characteristics. In model 3, we added variables with P values <0.05 in univariate analysis for adjustment (these included age per 1 year, weight per 1 kg decrement, CD4 count per 1 /μl decrement, HIV viral load per log10/ml, serum creatinine per 1 mg/dl, concurrent use of nephrotoxic drug(s), hepatitis B infection, and diabetes mellitus). The eGFR and the BMI were excluded from multivariate analysis because of their multicollinearity with age and serum creatinine, and weight, respectively, since eGFR and BMI are gained by the equation of those variables [22,26]. We chose to add weight instead of BMI because our previous work showed that weight was more useful and handy information to estimate the risk for TDF-related nephrotoxicity than BMI [16].

As a sensitivity analysis, creatinine clearance was similarly calculated with Cockcroft-Gault equation for each patient, creatinine clearance = [(140 - age) × weight (kg)] / (serum creatinine×72)(×0.85 for females) [27]. Actual body weight was used for the calculation. The impact of TDF use over ABC for >25% decrement of creatinine clearance from the baseline was estimated in univariate analysis and multivariate analysis adjusted with the before mentioned variables with Cox proportional hazards model.

To estimate the impact of weight on TDF-related nephrotoxicity, we did subgroup analysis for intertertile baseline body weight categories: ≤60, 61–68, and >68 kg. Then, the abovementioned multivariate analysis with eGFR was conducted for each subgroup.

We also used a repeated measures mixed model to estimate and compare changes in eGFR between ABC and TDF from baseline to 2 years after initiation of ART by 6-month intervals adjusted for baseline eGFR and weight [10]. For each patient, the eGFR values at closest to and preceding 24, 48, 72 and 96 weeks after commencement of ART were collected. In this analysis, censoring occurred at discontinuation of TDF or ABC, leaving care, or reaching the end of the observation period before 96 weeks. Sensitivity analysis with creatinine clearance calculated by Cockcroft-Gault equation was similarly conducted.

Statistical significance was defined at two-sided p values <0.05. We used hazard ratios (HRs) and 95% confidence intervals (95% CIs) to estimate the impact of each variable on renal dysfunction. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

The study subjects were 199 patients in the TDF arm and 304 patients in the ABC arm who fulfilled the abovementioned criteria. Table 1 shows the demographics, laboratory data, and medical conditions of the study population at baseline. The majority of the study population was males, comparatively young and had a small stature (median weight, 64 kg, median BMI, 22.2 kg/m^2). More than 80% of the patients in the two arms had ritonavir-boosted PI. In the ABC arm, patients had significantly lower CD4 count (p = 0.006), were significantly more likely to have hypertension (p<0.001), and tended to use more nephrotoxic drugs (p = 0.109). On the other hand, in the TDF arm, patients had marginally higher baseline eGFR (p = 0.098) and were significantly more likely to have hepatitis B virus infection (P<0.001). However, all other major background parameters were similar in the two groups (Table 1).

More than 25% decrement in eGFR from baseline occurred in 44 patients (22.1%) in the TDF arm and 41 (13.5%) in the ABC arm, with an estimated incidence of 9.84 and 4.55 per 100 person-years, respectively. Figure 1 shows the time from ART initiation to >25% decrease in eGFR by the Kaplan Meier method in the two groups. Patients who started TDF-containing ART were significantly more likely to develop renal dysfunction, compared to the ABC group (p = 0.001, Log-rank test). The median time from commencement of ART to occurrence of >25% decrement in eGFR was 246 days (range, 1–1,339 days) for the TDF arm and 501 days (range, 7–2,022) for ABC arm. The total observation period was 447.2 patient-years [median, 839 days, interquartile range (IQR), 357–1137 days] for the TDF arm and 901.7 patient-years (median, 1,119 days, IQR, 660.5–1509 days) for the ABC arm.

Univariate analysis showed a significant relationship between TDF use and >25% decrement in eGFR (HR = 1.747; 95%CI, 1.152–2.648; p = 0.009) (Table 2). Furthermore, old age, small body weight, low baseline CD4 count, high HIV viral load, high eGFR, low serum creatinine, concurrent use of nephrotoxic drugs, hepatitis B infection, and diabetes mellitus were associated with renal dysfunction. On the other hand, concurrent use of ritonavir boosted PIs was not associated with renal dysfunction (HR = 1.220; 95%CI, 0.663–2.244; p = 0.523). Multivariate analysis identified TDF use as a significant risk for >25% decrement in eGFR after adjustment for age and weight (adjusted HR = 1.893; 95%CI, 1.243–2.881; p<0.003) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR = 2.080; 95%CI, 1.339–3.232; p<0.001) (Table 3, Model 3). We also conducted a sensitivity analysis using BMI decrement instead of weight as a variable in Table 3, Model 3. The results were almost identical; TDF use over ABC use was a risk for renal dysfunction (adjusted HR 1.957, 95% CI 1.262–3.036, p = 0.003).

Sensitivity analysis with creatinine clearance confirmed the abovementioned findings: both univariate and multivariate analyses showed that TDF use was significantly associated with >25% decrement in eGFR (univariate analysis: HR = 2.212; 95%CI, 1.340–3.653; p = 0.002) (multivariate analysis: adjusted HR = 2.544; 95%CI, 1.493–4.335; p = 0.001).

Subgroup analysis of the patients stratified by intertertile baseline body weight showed that the lower the baseline body weight, the more evident the impact of TDF on renal dysfunction (≤60 kg: adjusted HR = 2.771; 95%CI, 1.494–5.139; p = 0.001) (61–68 kg: adjusted HR = 1.908; 95%CI, 0.764–4.768; p = 0.167) (>68 kg: adjusted HR = 0.997; 95%CI, 0.318–3.121; p = 0.995) (Table 4). These findings suggest that there is the effect modification by baseline body weight on TDF-associated renal dysfunction.

Data analysis by repeated measures mixed models showed a significant decrease in adjusted mean eGFR from the baseline to 96 weeks in both groups (TDF: -9.984 ml/min/1.73m^2, 95%CI -12.05 to -7.914 ml/min/1.73m^2, p<0.001; ABC: -5.393 ml/min/1.73m^2, 95%CI -7.087 to -3.699 ml/min/1.73m^2, p<0.001) (Figure 2). There was a statistically significant interaction between the two arms over time (p = 0.003), indicating that adjusted mean eGFR decreased more significantly in the TDF group than in the ABC group after initiation of ART. Analysis of eGFR in each group demonstrated a rapid decrease during the first 24 weeks,

Table 1. Baseline demographics and laboratory data of patients who received tenofovir- and abacavir-based antiretroviral therapy (n = 503).

	TDF (n = 199)	ABC (n = 304)	P value
Sex (male), n (%)	196 (98.5)	296 (97.4)	0.539
Median (IQR) age	36 (31–44)	37 (31–43)	0.436
Median (IQR) weight (kg)	64 (58–69)	64 (58.0–70.9)	0.426
Median (IQR) BMI (kg/m^2)	22.1 (20.4–23.9)	22.2 (20.3–24.6)	0.321
Median (IQR) eGFR (ml/min/1.73m^2)	119.4 (103.0–135.0)	115.6 (102.4–132.2)	0.098
Median (IQR) serum creatinine (mg/dl)	0.74 (0.67–0.84)	0.75 (0.68–0.83)	0.250
Median (IQR) CD4 count (/μl)	199 (109–272)	178.5 (75.3–234.8)	0.006
Median (IQR) HIV RNA viral load (log10/ml)	4.63 (4.20–5.20)	4.74 (4.23–5.20)	0.731
Ritonavir-boosted protease inhibitors, n (%)	173 (86.9)	256 (84.2)	0.441
Protease inhibitors (unboosted), n (%)	5 (2.5)	20 (6.6)	0.038
NNRTIs, n (%)	16 (8.0)	26 (8.6)	0.848
INIs, n (%)	5 (2.5)	2 (0.7)	0.119
Hypertension, n (%)	5 (2.5)	53 (17.4)	<0.001
Dyslipidemia, n (%)	4 (2.0)	4 (1.3)	0.718
Diabetes mellitus, n (%)	8 (4.0)	12 (3.9)	1.000
Concurrent use of nephrotoxic drugs, n (%)	65 (32.7)	121 (39.8)	0.109
Hepatitis B, n (%)	35 (17.6)	9 (3.0)	<0.001
Hepatitis C, n (%)	7 (3.5)	7 (2.3)	0.421
Current smoker, n (%)	93 (46.7)	149 (49.3)	0.585

TDF: tenofovir, ABC: abacavir, IQR: interquartile range, BMI: body mass index, eGFR: estimated glomerular filtration rate, NNRTI: non- nucleoside reverse transcriptase inhibitor, INI: integrase inhibitor.
doi:10.1371/journal.pone.0029977.t001

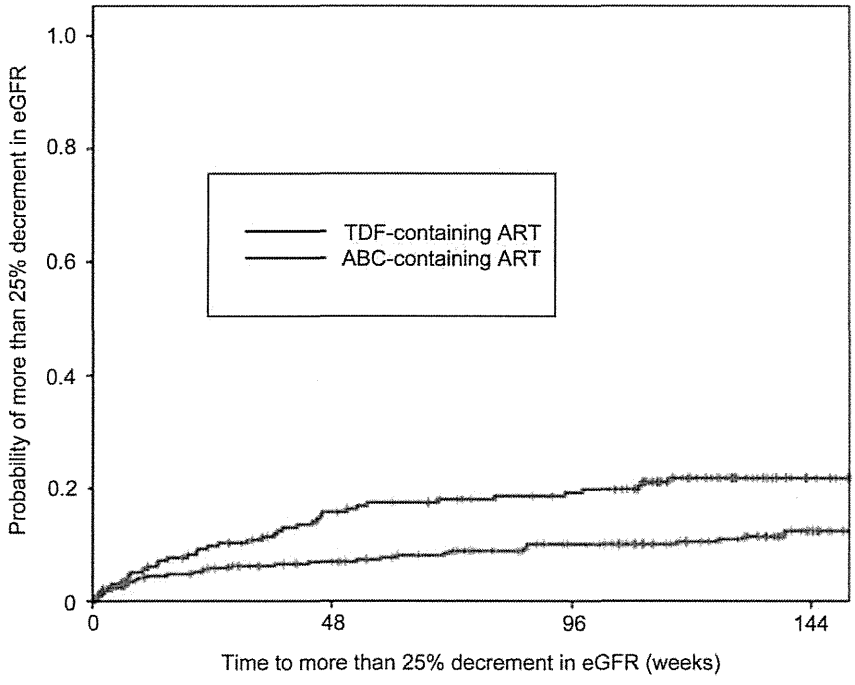

Figure 1. Kaplan-Meier curve showing the time to renal dysfunction in patients treated with TDF or ABC. Compared to treatment-naïve patients who commenced treatment with ABC, those on TDF were more likely to develop >25% fall in eGFR (p = 0.001, Log-rank test). TDF: tenofovir, ABC: abacavir, ART: antiretroviral therapy, eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0029977.g001

Table 2. Univariate analysis to estimate the risk of various factors in inducing more than 25% fall in eGFR.

	Hazard ratio	95% CI	P value
TDF vs. ABC use	1.747	1.152–2.648	0.009
Female gender	0.048	0.000–16.93	0.310
Age per 1 year	1.031	1.011–1.051	0.002
Weight per 1 kg decrement	1.047	1.023–1.072	<0.001
BMI per 1 kg/m^2 decrement	1.152	1.066–1.244	<0.001
CD4 count per 1 /μl decrement	1.006	1.004–1.008	<0.001
HIV viral load per log10/ml	1.562	1.179–2.071	0.002
Ritonavir-boosted protease inhibitors	1.220	0.663–2.244	0.523
Baseline eGFR per 1 ml/min/1.73m^2	1.009	1.005–1.014	<0.001
Baseline serum creatinine per 1mg/dl	0.016	0.003–0.086	<0.001
Concurrent nephrotoxic drug	2.134	1.417–3.214	<0.001
Hepatitis B	1.866	1.038–3.356	0.037
Hepatitis C	1.721	0.631–4.695	0.289
Diabetes mellitus	2.558	1.181–5.540	0.017
Hypertension	0.865	0.448–1.669	0.664
Current smoking	0.989	0.657–1.489	0.958

eGFR: estimated glomerular filtration rate, CI: confidence interval, TDF: tenofovir, ABC: abacavir, BMI: body mass index.
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followed by a plateau until 96 weeks. In sensitivity analysis with creatinine clearance calculated by Cockcroft-Gault equation, the result was the same; a significant decrease from the baseline to 96 weeks in both groups (TDF: -10.62 ml/min, 95%CI -13.78 to -7.458 ml/min; ABC: -4.325 ml/min, 95%CI -6.893 to -1.756 ml/min) and significantly more eGFR decrement in the TDF group ($p = 0.019$).

Discussion

In this observational Japanese cohort, treatment-naïve patients who started TDF-containing ART experienced eGFR decline of >25% approximately twice as likely compared to those treated with ABC-containing regimen. Univariate and multivariate analyses identified TDF use as an independent risk factor for renal dysfunction. Subgroup analysis showed that the effect of TDF on renal dysfunction was more evident in patients with lower body weight. Furthermore, eGFR decrement was significantly larger in the TDF group than in ABC group over the 2-year observation period.

In our previous study, we demonstrated a high incidence of TDF-associated nephrotoxicity in patients with low body weight, and the use of a robust statistical model indicated a greater decline in renal function in patients of low body weight treated with TDF [16]. The results of the present study further emphasize the importance of low body weight as a risk factor for TDF-related nephrotoxicity by showing that in a cohort of patients with low body weight, the incidence of renal dysfunction was twice higher with TDF use than with ABC use.

Among the studies designed to compare renal function after the commencement of TDF and ABC-containing ART for treatment-naïve patients, our cohort had the lowest median body weight (64 kg). This is lower than the median body weight of patients of the ASSERT study conducted in European countries (72 kg) [10]. The

Table 4. Multivariate analysis to estimate the risk of TDF- over ABC-based antiretroviral therapy in the induction of more than 25% fall in eGFR according to baseline body weight.

	Adjusted HR	95% CI	P value
Baseline body weight ≤60 kg (n = 171)			
TDF vs. ABC use	2.771	1.494–5.139	0.001
Baseline body weight 61–68 kg (n = 167)			
TDF vs. ABC use	1.908	0.764–4.768	0.168
Baseline body weight >68 kg (n = 165)			
TDF vs. ABC use	0.997	0.318–3.121	0.995

TDF use was adjusted with the same variables indicated in Model 3, Table 3: age per 1 year, weight per 1 kg decrement, CD4 count per 1 /μl decrement, HIV viral load per log10/ml, serum creatinine per 1 mg/dl, concurrent use of nephrotoxic drugs, hepatitis B infection, and diabetes mellitus.
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Table 3. Multivariate analysis to estimate the risk of TDF- over ABC-based antiretroviral therapy in inducing more than 25% fall in eGFR.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95% CI	HR	95%CI	HR	95%CI
TDF vs. ABC use†	1.747	1.152–2.648	1.893	1.243–2.881	2.080	1.339–3.232
Age per 1 year			1.029	1.010–1.048	1.020	1.000–1.040
Weight per 1 kg decrement†			1.046	1.022–1.071	1.028	1.005–1.052
CD4 count per 1 /μl decrement†					1.004	1.002–1.007
HIV viral load per log10/ml					1.048	0.749–1.466
Serum creatinine per 1 mg/dl†					0.053	0.009–0.304
Use of nephrotoxic drug					1.309	0.825–2.077
Hepatitis B					1.070	0.573–2.000
Diabetes mellitus					1.565	0.684–3.582

†P<0.05 in Model 3.
TDF: tenofovir, ABC: abacavir, eGFR: estimated glomerular filtration rate, HR: hazard ratio, CI: confidence interval.
doi:10.1371/journal.pone.0029977.t003

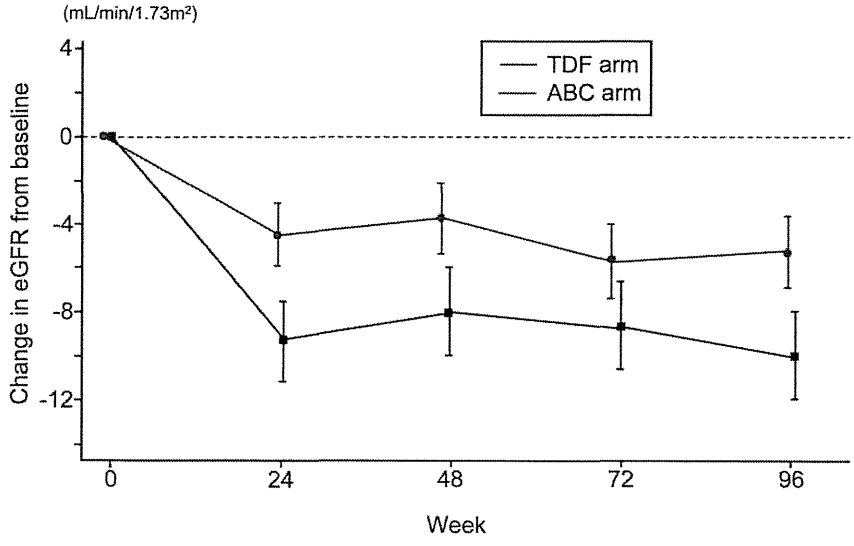

Figure 2. Changes in eGFR in patients treated with TDF or ABC between baseline and 96 weeks. The fall in eGFR was significantly greater in the TDF group than the ABC group ($p = 0.003$). Data are adjusted mean ±95% confidence interval. eGFR: estimated glomerular filtration rate, TDF: tenofovir, ABC: abacavir.
doi:10.1371/journal.pone.0029977.g002

results of the present study on TDF-related nephrotoxicity differ from the findings of randomized clinical trials that demonstrated no major change in renal function of TDF- and ABC-treated patients over 48–96 week follow-up [2,10,11]. The discrepant results might arise from differences between observational cohort and clinical trials, since observational studies tend to express the results in "real world setting" whereas clinical trials include patients who fulfill more strict criteria, therefore with better profile [9]. The discrepant results could be also due to the use of different definitions for renal dysfunction in these studies. However, the discrepant results could also reflect the difference in median body weight between the present study and these clinical trials. The results of our subgroup analysis support this hypothesis by showing that the effect of TDF on renal dysfunction was more evident in patients with low body weight. Apart from being low-body-weighted, the patients in this study did not appear to have many of other established risks for TDF-related nephrotoxicity; they were comparatively young, had relatively stable CD4 count, and had only a few co-morbidities (Table 1). Although the majority concurrently used ritonavir-boosted PIs, which are a probable risk for TDF-related nephrotoxicity, ritonavir-boosted PIs were not significantly associated with renal dysfunction in our cohort (Table 2) [24].

Changes in eGFR in those patients treated with TDF-containing ART were characterized by a rapid decline during the first 24 weeks of therapy, followed by a plateau until 96 weeks (Fig. 2). This finding is consistent with that reported from the Johns Hopkins group [9,28]. Together with the finding that the median time from commencement of ART to the >25% decline in eGFR in the TDF-treated patients was 246 days, these results suggest that careful monitoring of renal function is particularly warranted in the first year of TDF-based therapy. Thus, we suggest that renal function should be monitored by measurement of serum creatinine at least once annually in resource-limited settings and twice annually in resource-rich settings in patients starting TDF-containing ART, especially those with baseline body weight <60 kg.

The Department of Health and Human Services guideline for the treatment of HIV infection in the U.S. lists ABC as an alternative NRTI because it can potentially cause serious hypersensitivity reaction and cardiovascular diseases (URL:http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). However, some international guidelines consider both TDF and ABC as the preferred NRTIs under the condition that ABC should be used with caution in patients with viral load >100,000 copies/mL, based on the low incidence of ABC-related hypersensitivity among HLA-B*5701-negative population and the controversial association between ABC and cardiovascular diseases [1,29–32] (URL: http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf) (http://www.haart-support.jp/guideline2011.pdf. in Japanese). The present study, together with our previous analysis that demonstrated preferential TDF-related nephrotoxicity in patients with low body weight, emphasize the advantage of ABC over TDF with regard to prognosis of renal function in low body weight patients [16].

TDF is the prodrug of acyclic nucleotide analog tenofovir, which is excreted by both glomerular filtration and active tubular secretion. Tenofovir is considered to cause mitochondrial damage in proximal renal tubular cells [33]. The concentration of tenofovir in the proximal renal tubules could be augmented with the complex interactions of pharmacological, environmental, and genetic factors, including small body weight, consequently resulting in renal tubular dysfunction [34]. Body weight has been identified as an important factor in TDF-related nephrotoxicity not only in clinical trials, but also in *in vitro* and pharmacokinetic studies [35–37].

The present study has several limitations. First, because of its retrospective nature, it was not possible to control the baseline characteristics of the enrolled patients. Thus, it is possible that patients with potential risk for TDF-related nephrotoxicity were not prescribed TDF. A proportion of patients treated with ABC had low CD4 count and others were hypertensive, both conditions are known risk factors for renal dysfunction [23,25]. However, for these reasons, the incidence of TDF-associated renal dysfunction might have been underestimated. Second, the definition of TDF-related nephrotoxicity, especially the criteria used to evaluate proximal renal tubular damage, is not uniformly established in the field and is different in the published studies. Accordingly, we

decided to adopt changes in eGFR, instead of parameters for proximal renal tubular damage. Using the eGFR as a marker for TDF-associated renal dysfunction, our results might have underestimated the incidence of TDF-related renal tubular dysfunction. However, the result of this study could be informative to resource-limited settings, where it is difficult to evaluate renal tubular markers. The rational and limitation of adopting more than 25% decrement in eGFR as the criterion for renal dysfunction were discussed in detail in our previous study [16]. Third, our cohort was characterized by the high prevalence of ritonavir-boosted PI use, which is considered by some groups a risk for TDF-related nephrotoxicity [24]. While it is difficult to completely exclude the impact of concurrent ritonavir-boosted PI in this study, it should be noted that the use of ritonavir-boosted PIs did not correlate with renal dysfunction in univariate analysis in this cohort (Table 2). Fourth, the study subjects were mainly men (mostly men who have sex with men and very few injection drug users). Further studies are needed to determine whether the findings of this study are also applicable to females, patients with different route of transmissions, and patients of different racial background.

In conclusion, the present study demonstrated a high incidence of renal dysfunction with TDF use, compared to ABC, among treatment-naïve patients with low body weight. TDF use was identified as an independent risk for renal dysfunction in a statistical model that included TDF as a primary exposure. At 96 weeks, patients with TDF showed greater eGFR decrement than patients treated with ABC. TDF is certainly a drug of choice in the treatment of HIV infection, but the importance of close monitoring of renal function in patients with small body weight, especially those with baseline body weight <60 kg should be emphasized for early detection of TDF-related nephrotoxicity. Further studies are warranted to elucidate the long-term prognosis of renal function with TDF use in patients with low body weight.

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Author Contributions

Conceived and designed the experiments: TN HK HG TS EK JT SO. Performed the experiments: TN HK TS TA KW EK MH. Analyzed the data: TN HK HG TS HH HY K. Tsukada MH K. Teruya YK. Contributed reagents/materials/analysis tools: TA KW HH JT HY K. Tsukada MH K. Teruya YK. Wrote the paper: TN HK HG TS EK SO.

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ORIGINAL ARTICLE

Hypertension and concomitant arteriosclerotic diseases are risk factors for colonic diverticular bleeding: a case–control study

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Abstract
Purpose Colonic diverticular bleeding is a major cause of lower gastrointestinal bleeding. However, a limited number of studies have been reported on the risk factors for diverticular bleeding. Our aim was to identify risk factors for diverticular bleeding.
Methods Our study design is a case (diverticular bleeding)–control (diverticulosis) study. We prospectively collected information of habits, comorbidities, history of medications and symptoms by a questionnaire, and diagnosed diverticular bleeding and diverticulosis by colonoscopy. Logistic regression models were used to estimate odds ratio (OR) and 95% confidence interval (CI).
Results A total of 254 patients (diverticular bleeding, 45; diverculosis, 209) were selected for analysis. Cluster (≥10 diverticula) type (OR, 4.0; 95% CI, 1.8–8.9), hypertension (OR, 2.2; 95% CI, 1.0–4.6), ischemic heart disease (OR, 2.4; 95% CI, 1.1–5.4), and chronic renal failure (OR, 6.4; 95% CI, 1.3–32) were independent risk factors for diverticular bleeding.
Conclusions Large number of diverticula, hypertension, and concomitant arteriosclerotic diseases including ischemic heart disease and chronic renal failure are risk factors for diverticular bleeding. This study identifies new information on the risk factors for diverticular bleeding.

Keywords Arteriosclerotic diseases · Colonic diverticular bleeding · Colonic diverticulosis · Risk factors

Abbreviations

CI	Confidence interval
GI	Gastrointestinal bleeding
NCGM	National Center for Global Health and Medicine
NSAIDs	Nonsteroidal anti-inflammatory drugs
OR	Odds ratio
PG	Prostaglandin
UMIN	University Hospital Medical Information Network

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Introduction

Colonic diverticula are pseudodiverticula resulting from herniation of the mucosa and submucosa through a weakened portion of the colonic wall [1]. Colonic diverticulosis is an acquired disease caused by increased intestinal pressure due to decreased intake of dietary fiber and mucosal fragility associated with aging [2–5]. The typical locations of colonic diverticula differ between the Western and Asian populations, appearing predominantly left-sided in the Western countries and predominately right-sided in Asia [6]. With changes in dietary habits and the aging of society, the incidence of colonic diverticulosis is expected to increase [3, 4].

Although colonic diverticulosis is usually asymptomatic, diverticulitis and diverticular bleeding can occasionally occur.