and femoral neck. Osteopenia and osteoporosis were defined using the World Health Organization (WHO) criteria. Normal BMD was defined as a t score of -1 or higher, osteopenia as a t score between -1 and -2.5, and osteoporosis as a t score of -2.5 or lower.[8] Age, body mass index (BMI), smoking habit, hemophilia, history of an AIDS-defined illness, nadir CD4 cell count, time with low CD4 cell count (<200 cell/μl), time on antiretroviral therapy (ART), TDF, and PI, were obtained by interview or medical records. Estimated glomerular filtration rate (eGFR) was calculated using the modified Modification of Diet in Renal Disease (MDRD) equation for Japanese populations.[9]

Statistical analysis

t scores and BMD of the lumbar spine and femoral neck were compared using Student's paired t-test. To determine the impact of independent variables, multivariate logistic regression analysis was used. In logistic regression analysis, the dependent variable was set as low BMD (t score lower than -1.0) at both the lumbar spine and femoral neck. We used the odds ratio (ORs) and 95% confidence interval (95% CI) to estimate the impact of each variable on low BMD.

To assess the impact of PI discontinuation, we compared the t scores between PI-experienced patients and patients who discontinued such therapy, using the Student's unpaired t-test. For evaluation of the correlation between the t score at the lumbar spine and the time on PI, ritonavir (RTV) at different dosage (100 mg/day and 200 mg/day), and other types of PI, Pearson's correlation coefficient was used. For further evaluation of the relationship between the time on TDF and BMD, we compared the t scores between those who were treated with PI plus TDF and those treated with PI only and had never been treated with TDF, using the Student's unpaired t-test. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Patient characteristics

The characteristics of the 184 study patients are summarized in Table 1. All patients underwent DXA for the lumbar spine and 164 underwent DXA for the femoral neck. Hemophiliacs constituted 36% ($n=67$) of the study subjects. Seventy-one patients (39%) had a history of infection with hepatitis C virus (HCV), including all 67 hemophiliacs. Among them, 16 of the 71 (23%) patients showed spontaneous viral clearance, 23 (32%) achieved sustained virologic response after antiviral therapy, and 2 (3%) patients were still on treatment and had undetectable levels of HCV viral load. The remaining 30 (45%) patients with chronic hepatitis C were nonresponders or never users of antiviral therapy. Among them, 9 (14%) had liver cirrhosis diagnosed by radiological findings. Although 41 (21%) patients had a history of AIDS-defined illness, 172 (93%) patients had been treated with ART and 148 (80%) patients had an undetectable level of HIV viral load.

The median durations of ART, PI, and TDF of the total population were 88, 38, and 23 months, respectively. Among 139 TDF-treated patients, the median time on TDF was 38 months (IQR 14–68 months). One hundred and forty-four patients had previously received PI-based treatment, and the numbers of patients who had been treated with each type of PI were 30 patients with nelfinavir (NFV), 47 with lopinavir (LPV/r), 34 with atazanavir (ATV), 21 with fosamprenavir (FPV) or amprenavir (APV), 74 with darunavir (DRV), 4 with indinavir (IDV), and 1 with saquinavir (SQV). The total number of patients who had received RTV was 137, and of these, 102 and 63 patients had been treated with RTV at 100 and 200 mg/day, respectively.

TABLE 1. CLINICOPATHOLOGICAL CHARACTERISTICS OF THE 184 STUDY PATIENTS

Sex, (male/female)	184/0
Age: median (IQR)	43 (38–51)
Body mass index (kg/m^2)	22 (20–24)
Hypertension, n (%)	42 (23%)
Current smoking, n (%)	99 (54%)
Hemophilia, n (%)	67 (36%)
History of AIDS-defined illness, n (%)	40 (22%)
Positive HBsAg, n (%)	8 (4%)
Positive HCV-Ab, n (%)	71 (37%)
Liver cirrhosis, n (%)	10 (5%)
Diabetes mellitus, n (%)	7 (4%)
Current CD4$^+$ T cell count (cells/μl)	493 (322–623)
Nadir CD4$^+$ T cell count (cells/μl)	141 (54–218)
Low CD4$^+$ T cell count (<200 cells/μl) for >1 year, n (%)	52 (28%)
Current suppressed viral load (<20 copies/ml), n (%)	148 (80%)
Current use of ART, n (%)	172 (93%)
Time on ART (months)	88 (26–153)
Current use of protease inhibitors, n (%)	117 (64%)
Never use of protease inhibitors, n (%)	40 (22%)
Time on protease inhibitors (months)	38 (2–81)
Current use of tenofovir, n (%)	114 (62%)
Never use of tenofovir, n (%)	45 (24%)
Time on tenofovir (months)	22 (0–60)
Serum creatinine (mg/dl)	0.78 (0.68–0.89)
Estimated glomerular filtration rate (ml/min/1.73 m^2)	86.0 (74.7–100.3)

Values are median (IQR) or number (%) of patients.
HBsAg, hepatitis B virus surface antigen; HCV-Ab, hepatitis C virus antibody; ART, antiretroviral therapy; ART, antiretroviral therapy.

Prevalence of low bone mineral density

Based on the WHO criteria, osteopenia and osteoporosis were diagnosed in 46% and 10% of the patients at the lumbar spine and 53% and 12% at the femoral neck, respectively. The mean t scores were -1.1 [standard deviation (SD) 1.1] for the lumbar spine and -1.4 (SD: 1.1) for the femoral neck (Fig. 1A). The mean BMD scores were 0.914 g/cm^2 (SD: 0.199 g/cm^2) at the lumbar spine and 0.694 g/cm^2 (SD: 0.221 g/cm^2) at the femoral neck (Fig. 1B). Both the t score and BMD at the femoral neck were significantly lower than those at the lumbar spine ($p=0.008$ for t score and $p<0.001$ for BMD).

Impact of related risk factors

In multivariate logistic analysis, statistically significant regression models were built for low BMD (t score <-1) at the lumbar spine ($p=0.038$) and at the femoral neck

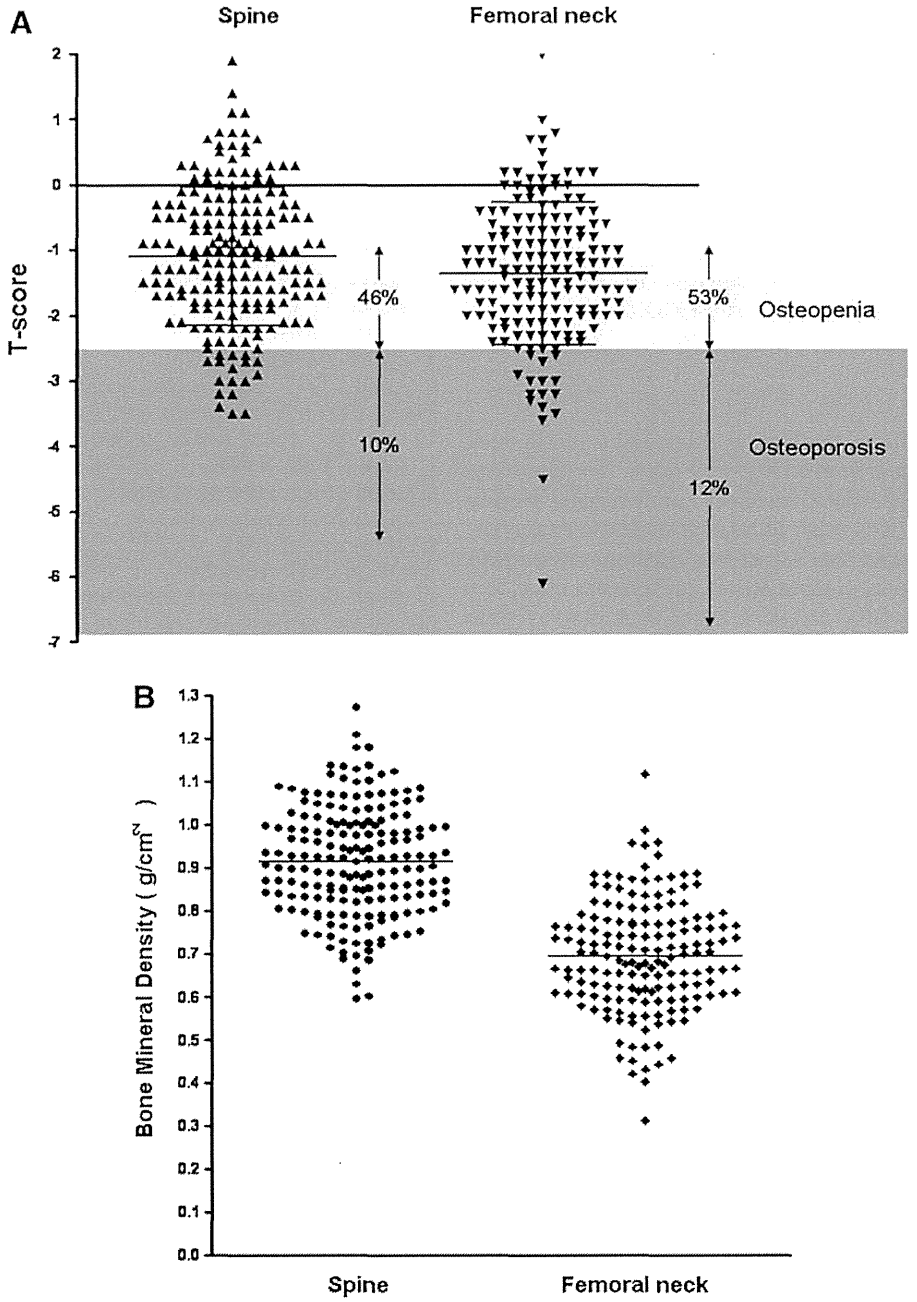

FIG. 1. **(A)** Distribution of *t* scores at lumbar spine and femoral neck. Light areas: osteopenia; dark gray areas: osteoporosis. **(B)** Distribution of bone mineral density (BMD) at lumbar spine and femoral neck. In both **(A)** and **(B)**, data are mean ± standard deviation. Differences in the mean scores of the spine and femoral neck were tested by the Student's paired *t*-test.

($p < 0.001$) (Table 2). In logistic analysis, the following factors were associated with low BMD at both the lumbar spine and femoral neck: longer duration of treatment with a PI [odds ratio (OR) 1.100 and 1.187 per 1 year increase of PI use and 95% confidence interval (CI) 1.003–1.207 and 1.043–1.351; $p = 0.042$ and 0.009, respectively] and lower body mass index [OR: 0.938 and 0.852, CI 0.892–0.992 and 0.783–0.927; $p = 0.024$ and < 0.001, respectively]. Low BMD at the femoral neck also correlated with age [OR: 1.071; CI 1.029–1.115; $p = 0.001$] and hemophilia [OR: 8.139; CI 2.594–25.337; $p < 0.001$].

Impact of PI use and discontinuation on bone mineral density

The *t* scores of both the spine and femoral neck were significantly lower in patients who received PI than in those who never used PI [−1.2 vs. −0.7 at the spine ($p = 0.02$) and −1.5 vs. −0.9 at the femoral neck ($p = 0.002$), respectively] (Fig. 2A). Moreover, patients who discontinued PI had a higher spine *t* score than those who currently used PI (−0.8 vs. −1.3, $p = 0.04$) and had a *t* score level comparable to those patients who never used PI (−0.8 in PI-discontinued patients

TABLE 2. RESULTS OF LOGISTIC ANALYSIS FOR BONE MINERAL ABNORMALITIES MEASURED FOR DIFFERENT JOINTS

	Univariate analysis			*Multivariate analysis*[a]		
	OR	*95% CI*	p *value*	*OR*	*95% CI*	p *value*
Low BMD at lumbar spine (t score < -1.0)						
Age (per 1 year increase)	1.015	0.986–1.045	0.309	1.016	0.989–1.042	0.249
Body mass index (per 1 increase)	0.924	0.845–1.011	0.086	0.938	0.892–0.992	0.024
Hemophilia	1.013	0.556–1.847	0.967			
Current smoking	1.690	0.942–3.302	0.078	1.651	0.903–2.971	0.104
History of AIDS-defined illness	1.630	0.800–3.323	0.176			
Nadir CD4 (per 1 increase of categories)						
≥350	1.000					
200–349	0.514	0.140–1.883	0.315			
≤199	0.799	0.241–2.653	0.714			
Time with CD4 <200/μl (per 1 year increase)	1.065	0.921–1.233	0.515			
Time on ART (per 1 year increase)	1.027	0.978–1.077	0.287	0.973	0.912–1.038	0.408
Time on TDF (per 1 year increase)	1.082	0.976–1.200	0.134	1.078	0.961–1.210	0.201
Time on PI (per 1 year increase)	1.081	1.009–1.159	0.026	1.100	1.003–1.207	0.042
Low BMD at femoral neck (t-score < -1.0)						
Age (per 1 year increase)	1.012	1.005–1.019	0.001	1.071	1.029–1.115	0.001
Body mass index (per 1 increase)	1.017	1.003–1.031	0.018	0.852	0.783–0.927	<0.001
Hemophilia	3.954	1.850–8.448	<0.001	8.139	2.594–25.337	<0.001
Current smoking	1.206	0.642–2.265	0.561	0.238	0.734–3.460	0.238
History of AIDS-defined illness	1.870	0.806–4.338	0.141	0.124	0.795–6.789	0.124
Nadir CD4 (per 1 increase of categories)						
≥350	1.000			1.000		
200–349	1.593	0.425–5.971	0.489	1.553	0.355–6.783	0.559
≤199	0.984	0.293–3.301	0.979	0.757	0.174–3.285	0.710
Time with CD4 <200/μl (per 1 increase of categories)	1.072	0.951–1.209	0.257	0.844	0.684–1.042	0.114
Time on ART (per 1 year increase)	1.070	1.034–1.117	<0.001	0.968	0.880–1.066	0.509
Time on TDF (per 1 year increase)	1.084	1.005–1.119	0.037	0.990	0.848–1.156	0.900
Time on PI (per 1 year increase)	1.151	1.079–1.225	<0.001	1.187	1.043–1.351	0.009

[a]In the analysis for lumbar spine, the final model obtained by backward stepwise elimination included the time on ART, TDF, and PI, current smoking, BMI, and age. OR, odds ratios; CI, confidence intervals; ART, antiretroviral therapy; TDF, tenofovir disoproxil fumarate; PI, protease inhibitors; BMD, bone mineral density.

vs. −0.7 in PI-never use patients, $p=0.97$) (Fig. 2B). In contrast, there was no significant difference in femoral neck t score between PI-discontinued patients and PI current-use patients (−1.3 vs. −1.5, $p=0.38$) or between PI-discontinued patients and PI-never use patients (−1.3 vs. −0.9, $p=0.24$) (Fig. 2C).

Impact of different types of PIs on bone mineral density

While the correlation between the duration of treatment of any PI and spine t score was significant ($r=-0.180$, $p=0.013$) (Fig. 3A), the duration of treatment with RTV showed a better correlation with spine t score (-0.207, $p=0.004$) (Fig. 3B). When both the time on RTV and the time on PI were entered as independent variables in logistic analysis for low BMD at the lumbar spine, a statistically significant model was built by elimination of the time on PI. In this model, the time on RTV was significantly associated with low BMD (OR: 1.146, 95% CI 1.032–1.273, $p=0.011$). At the femoral neck, RTV was associated with low BMD (OR: 1.267 per 1 year increase of RTV, 95% CI 1.010–1.589, $p=0.041$), whereas the time on PI was not (OR: 0.983 per 1 year increase of PI, 95% CI 0.803–1.202, $p=0.864$). There were no significant correlations between spine t score and the duration of treatment with RTV at either 100 mg/day ($r=-0.134$, $p=0.071$) (Fig. 3C) or 200 mg/day ($r=-0.133$, $p=0.073$) (Fig. 3D). No significant correlations were found between different types of PIs and spine t score (NFV: $r=-0.023$, $p=0.758$; LPV/r: $r=-0.080$, $p=0.239$; DRV: $r=-0.069$, $p=0.355$; ATV: $r=-1.123$, $p=0.097$; FPV or APV: $r=0.091$, $p=0.218$).

Comparison of BMD between PI- and PI-TDF-treated patients

For further confirmation of the poor association between TDF use and BMD loss, t scores were compared between patients who had been treated with both PI and TDF ($n=118$) and patients who received PI-based treatment and had never been treated with TDF ($n=26$). Neither spine nor femoral neck t scores were significantly different between the two groups (PI + TDF: −1.2, PI alone: −1.0, $p=0.414$ for spine t score, −1.5 vs. −1.5, $p=0.844$ for femoral neck, respectively).

Discussion

The present study showed that for Asian HIV-infected patients, PI use was the most significant determinant of low BMD at both the spine and femoral neck. Moreover, our logistic regression models strongly suggested that long-term use of PI has a gradual and cumulative effect on BMD.

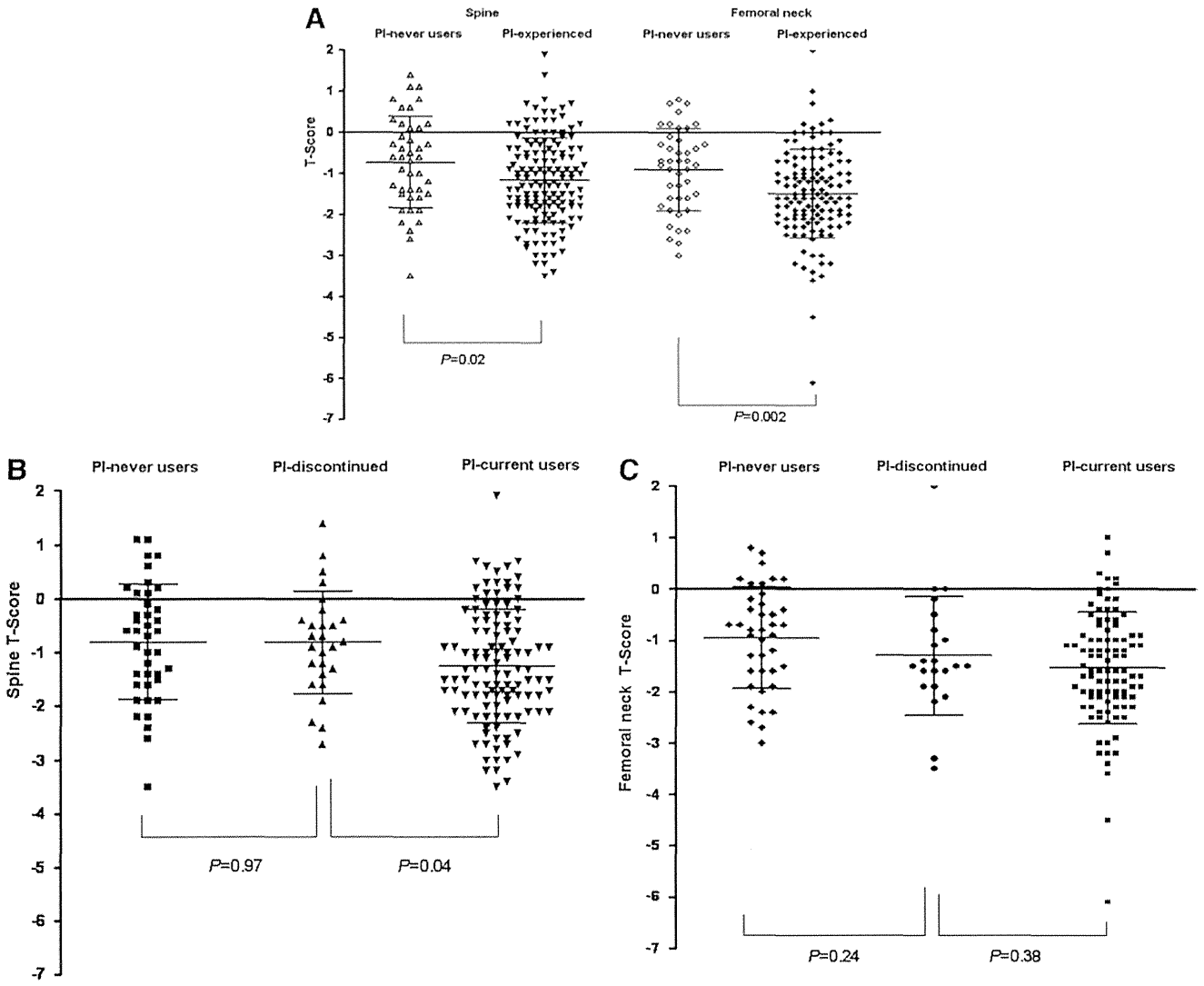

FIG. 2. **(A)** Comparison of *t* scores at lumbar spine and femoral neck between patients who were treated and never treated with a protease inhibitor (PI). Comparison of *t* score at lumbar spine **(B)** and at femoral neck **(C)** among patients who never used PI (left), discontinued PI (center), and are currently using PI (right). Data are mean ± standard deviation.

Although large cohort studies have already shown that PI use can cause BMD loss,[4–6] it still remains unclear which type of PI causes BMD loss. Our study found no significant association between the use of any particular type of PI and BMD loss, which is consistent with a previous *in vitro* study that evaluated the impact of different PIs on osteoblast activity using an osteoblast-like cell line.[10] Both *in vitro*[11,12] and *ex vivo* studies[13] reported that RTV promotes the proliferation/activation of osteoclasts, causing increased bone absorption. Our study added support to previous studies that RTV plays a major role in PI-associated BMD loss,[13] although there is insufficient data to conduct direct a comparison of BMD between patients treated with unboosted and boosted PI. The correlations between the two different dosages of RTV and BMD were almost comparable levels of strength, suggesting that RTV can cause BMD loss not dose dependently but time dependently irrespective of the dose. However, at this stage, we recommend further evaluation of the effect of each type of PI, since the subanalyses conducted in the present study have limited power for cause–effect evaluation due to the relatively small number of patients treated with certain types of PI.

Does discontinuation of PI lead to recovery of BMD? It seems there is no definitive answer to this question. A small cohort substudy showed possible BMD recovery after switching PI to raltegravir.[14] However, the change in BMD after switching was too small in that study to confirm the recovery effect of PI discontinuation. The present study provides additional data in support of a lower decrease in BMD by showing a large difference in BMD between PI-discontinued and -continued patients, although it is a cross-sectional study. A prospective longitudinal cohort study using a larger population on longer use of PI is necessary for a more precise evaluation of the reversibility of PI-associated BMD loss. It should be noted that the PI-discontinued patients showed a higher BMD level not in the femoral neck but in the lumbar spine, which is consistent with some large cohort study showing that PI causes greater BMD loss in the lumbar spine than the femoral neck.[4,5] This interesting discrepancy is well explained by the difference in bone tissue

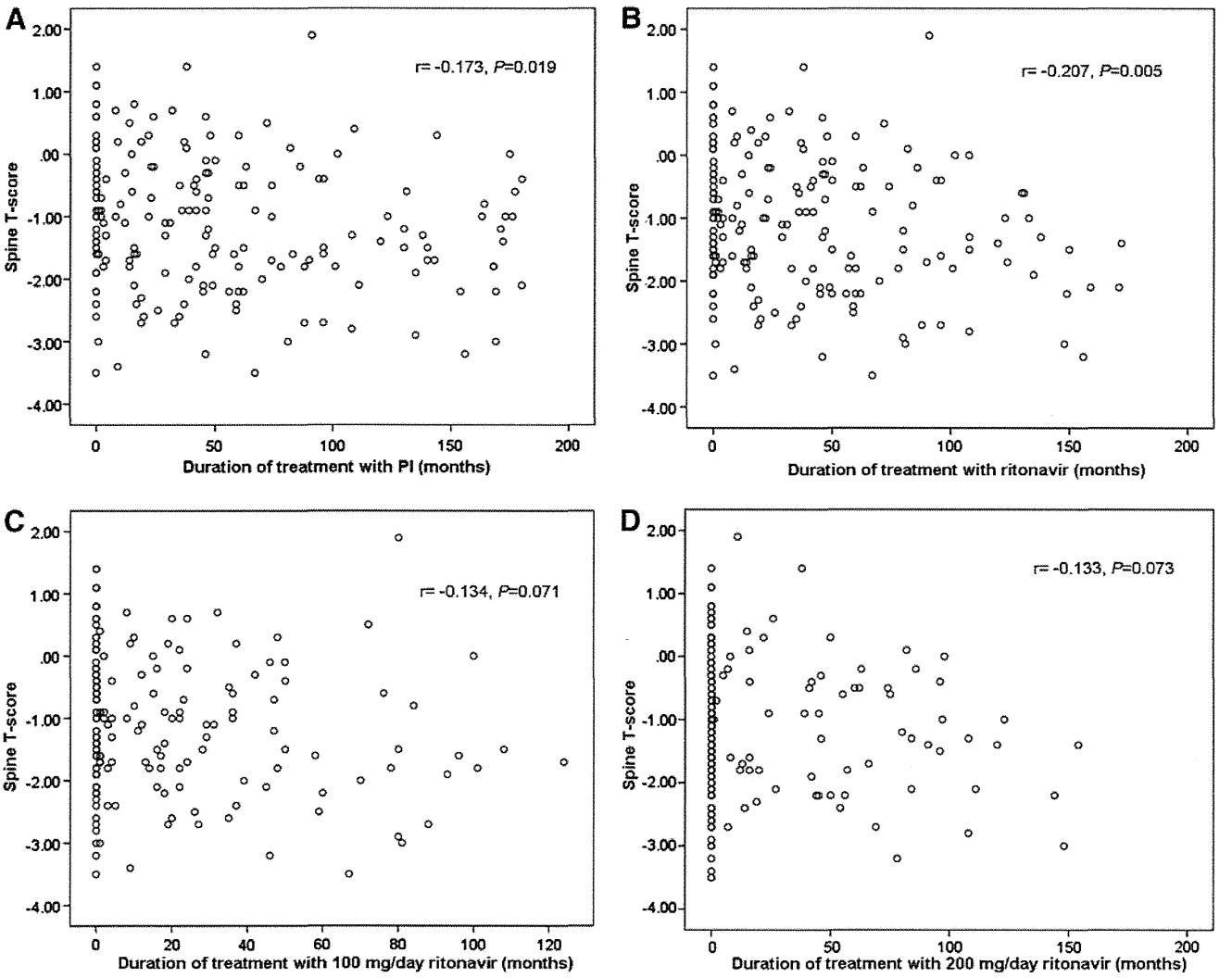

FIG. 3. Scattered dot plots of the correlation between *t* score at lumbar spine and duration of treatment with PI **(A)**, ritonavir (RTV) **(B)**, RTV at 100 mg/day **(C)**, and RTV at 200 mg/day **(D)**. Correlations were tested by Pearson's correlation coefficient.

type between the vertebrae and femur. While the femur contains abundant cortical substance with few osteoclasts, the vertebrae comprise osteoclast-rich trabecular substance. Therefore, discontinuation of osteoclast-activating agents, such as RTV, can cause a slower decrease of BMD in vertebrae compared with the femur.

TDF can cause BMD loss mainly through persistent urinary loss of phosphates.[4,7,15,16] However, our study did not show any significant association between TDF use and low BMD. While the exact reason for this finding is not clear, it could be related to the general clinical practice in Japan: TDF is often discontinued in Japan upon identification of modest proximal tubular dysfunction (a low level of percent tubular reabsorption of phosphates or a high level of urine-β_2-microglobulin) in HIV-infected patients.[16,17] This practice is an important limitation in the present study.

Hemophilia is a risk factor for BMD loss based on the associated hemophilic arthropathy and long-term disuse.[18,19] However, the present study demonstrated an almost equal prevalence of spine BMD abnormalities in hemophiliacs and HIV-infected patients [rate of osteoporosis, hemophiliacs: 5/67 (7%), other patients: 14/117 (12%); rate of osteopenia, hemophiliacs: 32/67 (48%), other patients: 52/117 (44%)]. Furthermore, the mean (standard deviation) *t* score of the lumbar spine was −1.1 (1.0) in hemophiliacs and −1.1 (1.1) in other patients. Thus, with regard to lumbar spine BMD, the present study well reflects the general Asian HIV-infected population. On the other hand, BMD abnormalities are common in hemophiliacs including abnormalities of the femoral neck [rate of osteoporosis, hemophiliacs: 15/57 (26%), other patients: 5/107 (5%); rate of osteopenia, hemophiliacs: 32/57 (56%), other patients: 56/107 (52%)]. The mean (standard deviation) *t* score of the femoral neck was −2.0 (1.1) in hemophiliacs and −1.0 (0.9) in other patients. Multivariate analysis identified age, BMI, and hemophilia as significant determinants of BMD at the femoral neck. Thus, BMD at the femoral neck is considered to be largely influenced by weight load and disuse.

In conclusion, long-term use of PI was identified as a significant risk factor for BMD loss in HIV-infected Asian patients. Furthermore, the results demonstrated that the negative effect of PI on BMD was time dependent. In particular, RTV plays a major role in PI-associated BMD loss irrespective of the dose. Discontinuation of PI seems to lessen the decrease in BMD, especially in the lumbar spine,

suggesting that withdrawal of PI is a promising option for treatment of BMD abnormalities.

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Author Disclosure Statement

No competing financial interests exist.

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Long-term exposure to tenofovir continuously decrease renal function in HIV-1-infected patients with low body weight: results from 10 years of follow-up

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Objectives: To investigate the effect of long-term tenofovir disoproxil fumarate (TDF) use on renal function, especially in patients with low body weight who are vulnerable to TDF nephrotoxicity.

Design: A single-center, observational study in Tokyo, Japan.

Methods: We performed a 10 years cohort study of 792 HIV-1-infected patients. The effect of long-term TDF use on estimated glomerular filtration rate (eGFR) was investigated on treatment-naive patients who started TDF-containing antiretroviral therapy ($n=422$) and those who started abacavir-containing antiretroviral therapy as control ($n=370$). Three renal endpoints were examined by the logistic regression model: decrement in eGFR of higher than 10 ml/min per 1.73 m^2 relative to the baseline, more than 25% decrement in eGFR, and eGFR lower than 60 ml/min per 1.73 m^2 at least 3 months apart. The loss in eGFR was estimated using linear mixed models for repeated measures.

Results: The median weight at baseline was 63 kg. TDF use increased the risk of all three renal outcomes compared with the control group: higher than 10 ml/min per 1.73 m^2 decrement in eGFR [adjusted odds ratio (OR) = 2.1, 95% confidence interval (CI) 1.45–3.14, $P<0.001$], more than 25% decrement (adjusted OR = 2.1, 95% CI 1.50–2.90, $P<0.001$), and eGFR lower than 60 ml/min per 1.73 m^2 at least 3 months apart (adjusted OR = 3.9, 95% CI 1.62–9.36, $P=0.002$). The cumulative mean loss relative to the control after 1, 2, 3, 4, and 5 years of TDF exposure was −3.8, −3.6, −5.5, −6.6, and −10.3 ml/min per 1.73 m^2, respectively, indicating that the loss in eGFR increased over time ($P<0.001$).

Conclusion: In this cohort of patients with low body weight, TDF exposure increased the risk of renal dysfunction. Furthermore, the loss in eGFR relative to the control increased continuously up to 5 years.

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Keywords: HIV-1, low body weight, renal dysfunction, tenofovir disoproxil fumarate, treatment-naive

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Introduction

Tenofovir disoproxil fumarate (TDF) is one of the most widely used nucleotide reverse transcriptase inhibitors (NRTIs) for the treatment of HIV-1 infection in both resource-rich and resource-limited settings [1,2], and also for the treatment of hepatitis B infection [3,4]. Furthermore, TDF, at a fixed dose with emtricitabine, has been approved in the United States for the prevention of sexual transmission of HIV-1 in uninfected adults as preexposure prophylaxis [5,6].

TDF is known to cause renal proximal tubular dysfunction [7–10] and also reduces estimated glomerular filtration rate (eGFR) more than other NRTIs [11–13]. To date, the extent of TDF-induced renal dysfunction is regarded as mild and tolerable [14,15], and one meta-analysis recommended that TDF use should not be restricted even when regular monitoring of renal function and serum phosphate levels is impractical [16]. Furthermore, although evidence is limited, most of the TDF-induced loss in renal function is considered to occur during the first year of exposure [12,15].

However, a large proportion of studies that investigated TDF nephrotoxicity were based on an analysis of a relatively short observation period, typically a few years, and little information is available on the effect of long-term TDF use on the prognosis of renal function. This is important as HIV-1 infection requires lifelong antiretroviral therapy (ART). In this regard, although small body weight is a well established risk factor for TDF nephrotoxicity [16,17], the TDF-related renal dysfunction 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 [17–20].

Based on the above background, the current study was designed to investigate the effects of long-term TDF use on renal function in HIV-1-infected patients with low body weight, using 10 years data from our observational cohort study.

Methods

Study design and patients

We performed a single-center cohort study of HIV-1-infected patients using the medical records at AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo. The effect of long-term TDF use on renal function was investigated on treatment-naive patients who started TDF-containing ART, and those who started abacavir (ABC)-containing ART as the control. ABC was chosen as the control because this NRTI is not known to be nephrotoxic and is not renally eliminated [21] and because the Japanese guidelines for the treatment of HIV-1 infection placed both TDF and ABC as the preferred NRTIs throughout the observational period [22]. The inclusion criteria were treatment-naivety, Japanese, age older than 17 years, and treatment with either the recommended 300 mg/day dose of TDF or 600 mg/day dose of ABC-containing standard ART (consisting of one nonnucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI) or integrase strand transfer inhibitor (INSTI), and two NRTIs) at our clinic between 1 January 2004 and 31 December 2011. Furthermore, the following exclusion criteria were applied: start of ART at other facilities, baseline eGFR of lower than 60 ml/min per 1.73 m^2, discontinuation of TDF or ABC within 90 days after initiation of ART, or start of ART with both TDF and ABC. Of the 1334 patients who started ART at our clinic during the study period, 792 patients fulfilled these criteria and constituted the study patients (see Figure, Supplemental Digital Content 1, http://links.lww.com/QAD/A537, which shows patient enrollment process). The study patients were followed up until 31 December 2013. Censoring occurred at discontinuation of TDF or ABC, referral to other hospitals, loss to follow-up, death, or end of the observation period. The inclusion of Japanese patients only served to examine a population with relatively small body stature, compared with whites and African Americans [17]. The selection of TDF or ABC at baseline was left to the discretion of the attending physician, because both drugs were the preferred NRTIs during the study period in the Japanese guidelines [22]. The attending physician also selected the key drug (NNRTI, PI, or INSTI). In Japan, TDF became available from April 2004 and ABC from September 1999.

The study was approved by the human research ethics committee of National Center for Global Health and Medicine. All patients included in this study provided written informed consent for their clinical and laboratory data to be used and published for research purposes. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Measurements

eGFR was calculated using the Japanese equation based on standardized serum creatinine, sex, and age, which was developed by the Japanese Society of Nephrology (JSN): $eGFR = 194 \times [\text{serum creatinine}]^{-1.094} \times [\text{age}]^{-0.287} \times$ [0.739 if woman] [23]. This equation was used because the Japanese equation performs better than The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [24] for patients with small body stature, such as Japanese, especially in individuals with GFR lower than 60 ml/min per 1.73 m^2 [25]. The 2013 practice guidelines for patients with CKD published by JSN also recommend the use of this equation for the Japanese, rather than CKD-EPI, which was derived mostly from whites and African Americans [25,26].

The baseline eGFR was estimated for each patient from age, sex, and serum creatinine measurements made closest to and preceding the commencement of ART by no more than 90 days. Patients visited our clinic at least every 3 months for monitoring $CD4^+$ cell count, HIV-1 viral load, and eGFR as the prescription period under the Japanese healthcare system is limited to 3 months. Thus, for calculation of follow-up eGFR value, we collected serum creatinine values measured closest to every 90 day within a range of 45 days from initiation of 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 [16,19,27,28]. They included age, sex, body weight, BMI = {body weight (kg)/[(height (m)]^2}, history of AIDS, route of HIV-1 transmission, 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 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 SBP higher than 140 mmHg or DBP higher than 90 mmHg at the clinic, dyslipidemia defined by current treatment with lipid-lowering agents, coinfection with hepatitis B defined by positive hepatitis B surface antigen, coinfection with hepatitis C defined by positive HCV viral load, and current smoking). At our clinic, body 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 180 days.

Statistical analysis

The primary exposure variable was TDF use over the control (ABC) as part of the initial ART. Three renal endpoints were applied in this study; we primarily focused on decrement in eGFR of higher than 10 ml/min per 1.73 m^2 relative to the baseline [29], because this endpoint is considered appropriate for patients with well maintained renal function, such as the study population; more than 25% decrement in eGFR relative to the baseline [17,18]; and two consecutive measurements of eGFR lower than 60 ml/min per 1.73 m^2 at least 90 days apart [30]. Changes in eGFR were plotted from the baseline measurement until occurrence of each of the three renal endpoints, and the logistic regression model was used to estimate the effect of TDF use over control on the occurrence of these renal endpoints. The model was adjusted for baseline eGFR, baseline body weight, nephrotoxic drug use, PI/r use, $CD4^+$ cell count, hypertension, dyslipidemia, and diabetes mellitus, which are established risk factors for TDF nephrotoxicity [13,16,27,28]. Baseline age was not added to the model to avoid over adjustment because the equation for eGFR calculation already includes age, and the baseline age was not associated with TDF use, indicating that age is not a confounding factor for the association between TDF use and eGFR. Furthermore, older age at baseline was shown to be a predictive variable for lower baseline eGFR (linear regression, $P < 0.0001$). In this case, adding predictive covariates to the logistic regression model will have detrimental effects on precision [31].

To investigate the effect of body weight on TDF-related nephrotoxicity, we did subgroup analysis for baseline weight categories: at least 70 kg and lower than 70 kg. Then, the multivariate logistic analysis for the renal endpoint of the occurrence of higher than 10 ml/min per 1.73 m^2 decrement in eGFR was conducted for each subgroup.

To further investigate the effect of TDF on renal function, we estimated the decrement in eGFR in the TDF group relative to the control group by calculating the difference in eGFR loss between the TDF and control group from baseline to 5 years after initiation of ART by 90 days intervals with a linear mixed models for repeated measures. We constructed the model with a random effect for patients. This model also included fixed effects for assigned treatment, baseline eGFR, baseline body weight, nephrotoxic drug use, PI/r use, $CD4^+$ cell count, hypertension, dyslipidemia, and diabetes mellitus. Interaction terms for time by treatment were included.

As additional analyses, the statistical analyses using eGFR calculated with CKD-EPI equation adjusted with the Japanese coefficient were also performed: eGFR = 0.813 (a Japanese coefficient) $\times$ 141 $\times$ min(SCr/κ, 1)$^{\alpha}$ $\times$ max(SCr/κ, 1)$^{-1.209}$ $\times$ 0.993$^{\text{Age}}$ $\times$ 1.018 (if female), where SCr is serum creatinine, κ is 0.7 for women and 0.9 for men, α is -0.329 for women and -0.411 for men, min indicates the minimum of SCr/κ or 1, and max indicates the maximum of SCr/κ or 1 [32].

Statistical significance was defined at two-sided $P < 0.05$. We used odds ratios (ORs) with 95% confidence intervals (95% CIs) as a measure of the effect of TDF use on renal endpoints. All statistical analyses were performed with SAS Software, version 9.3 (SAS Institute, Cary, North Carolina, USA).

Results

Of the 792 study patients, 422 patients started TDF-containing ART (TDF group) whereas the remaining 370 patients formed the control group who started ABC-containing ART (see Figure, Supplemental Digital

Table 1. Baseline characteristics of patients who started tenofovir disoproxil fumarate-containing antiretroviral therapy and controls (abacavir-containing antiretroviral therapy).

	Study patients ($n=792$)	TDF ($n=422$)	Control (ABC) ($n=370$)	P
Sex (male), *n* (%)	769 (97)	412 (98)	357 (97)	0.40
Age[a]	36 (31–43)	36 (31–43)	36 (31–44)	0.23
Weight (kg)[a]	63 (57.8–70.4)	62.9 (57.2–69.8)	63.8 (58.0–71.4)	0.25
BMI (kg/m^2)[a]	22 (20.1–24.1)	21.9 (20.1–23.8)	22.2 (20.3–24.6)	0.23
eGFR (ml/min per 1.73 m^2)[a]	95.7 (84–110)	96.5 (84.7–111.5)	95.4 (83.7–108.6)	0.32
Serum creatinine (mg/dl)[a]	0.74 (0.66–0.82)	0.73 (0.66–0.82)	0.74 (0.67–0.83)	0.27
CD4$^+$ cell count (/μl)[a]	189 (78–266)	199 (85–281)	183 (73–241)	0.002
HIV RNA viral load ($\log_{10}$/ml)[a]	4.76 (4.26–5.23)	4.76 (4.26–5.23)	4.76 (4.27–5.26)	0.93
Ritonavir-boosted protease inhibitors, *n* (%)	673 (85)	368 (87)	305 (82)	0.073
Protease inhibitors (unboosted), *n* (%)	28 (4)	8 (2)	20 (5)	0.011
NNRTIs, *n* (%)	48 (6)	20 (5)	28 (8)	0.10
INSTIs, *n* (%)	45 (6)	28 (7)	17 (5)	0.22
Hypertension, *n* (%)	118 (15)	41 (10)	77 (21)	0.001
Dyslipidemia, *n* (%)	9 (1)	5 (1)	4 (1)	1.00
Diabetes mellitus, *n* (%)	29 (4)	9 (2)	20 (5)	0.021
Concurrent use of nephrotoxic drugs, *n* (%)	218 (28)	88 (21)	130 (35)	<0.001
Hepatitis B, *n* (%)	62 (8)	57 (14)	5 (1)	<0.001
Hepatitis C, *n* (%)	37 (5)	20 (5)	17 (5)	1.00
History of AIDS, *n* (%)	183 (23)	89 (21)	94 (25)	0.15
Homosexual contact, *n* (%)	689 (87)	364 (86)	325 (88)	0.94
Current smoker, *n* (%)	369 (47)	193 (46)	176 (48)	0.57
ART duration (years)[a]	3.52 (2.29–5.18)	3.19 (2.20–4.67)	4.59 (2.48–5.18)	<0.001

ABC, abacavir; ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; TDF, tenofovir disoproxil fumarate.
[a]Median (interquartile range).

Content 1, http://links.lww.com/QAD/A537, which shows patient enrollment process). Table 1 shows the characteristics of the study population at baseline. The majority of the study population was men, comparatively young, and had a small body stature [median weight, 63 kg (interquartile range [IQR] 57.8–70.4 kg), median BMI 22.0 kg/m^2 (IQR 20.1–24.1)]. There was no difference in baseline eGFR between the two groups ($P=0.32$). More than 80% of the patients of the two groups used PI/r. Patients of the TDF group had higher CD4$^+$ cell count ($P=0.002$) and were less likely to have hypertension ($P=0.001$), diabetes mellitus ($P=0.021$), and on concurrent nephrotoxic drugs ($P<0.001$), than the control. The median duration of ART was longer in the control group [median, 1675 days, interquartile range (IQR), 904–1890 days] than in the TDF group [median, 1164 days, IQR, 802–1705 days) ($P<0.001$)]. The total observation period was 1347.5 patient-years for the TDF group and 1379.3 patient-years for the controls.

During the observation period, an eGFR decline from baseline of higher than 10 ml/min per 1.73 m^2 occurred in 348 (82.5%) of the TDF group and 265 (71.6%) of the control group (TDF use over control: adjusted OR 2.1, 95% CI 1.45–3.14, $P<0.001$) (Table 2). Furthermore, higher baseline eGFR, higher CD4$^+$ cell count also increased the risk of higher than 10 ml/min per 1.73 m^2 decrement in eGFR.

More than 25% decrement in eGFR occurred in 172 (40.8%) patients of the TDF group and 97 (26.2%) of the control (adjusted OR = 2.1, 95% CI 1.50–2.90, $P<0.001$) (Table 3), and two consecutive measurements of eGFR lower than 60 ml/min per 1.73 m^2 were encountered in 26 (6.2%) patients of the TDF group and in 14 (3.8%) of the control (adjusted OR = 3.9, 95% CI 1.62–9.36, $P=0.002$) (Table 4).

Subgroup analysis by baseline body weight above and below 70 kg showed that among patients with body weight at least 70 kg, TDF use relative to the control marginally increased the risk of higher than 10 ml/min per 1.73 m^2 decrement in eGFR (adjusted OR = 1.7, 95% CI 0.83–3.29, $P=0.15$), whereas among patients weighing lower than 70 kg, the effect of TDF use was more evident (adjusted OR = 2.5, 95% CI 1.55–4.00, $P<0.001$) than that among the entire study population (see Table 1, Supplemental Digital Content 2, http://links.lww.com/QAD/A537, which shows effects of initiating TDF-containing ART over control on higher than 10 ml/min per 1.73 m^2 decrement in eGFR according to baseline body weight).

Figure 1 shows the results of the linear mixed models for repeated measures up to 5 years. The adjusted cumulative mean loss increased continuously over the years in both the TDF and control groups: in TDF group, from −11.8 ml/min per 1.73 m^2 at 1 year of TDF to −23.7 ml/min per 1.73 m^2 at 5 years of TDF exposure, and in the control, from −8.0 ml/min per 1.73 m^2 at 1 year to −13.5 ml/min per 1.73 m^2 at 5 year of ART exposure. The adjusted cumulative mean loss in the TDF group

Table 2. Effects of initiating tenofovir disoproxil fumarate-containing antiretroviral therapy over control on >10 ml/min per 1.73 m^2 decrement in estimated glomerular filtration rate: multivariate logistic regression analysis.

	Adjusted OR	95% CI	*P*
TDF use relative to the control	2.1	1.45–3.14	<0.001
Baseline eGFR per 1 ml/min per 1.73 m^2 increment	1.1	1.05–1.08	<0.001
Weight per 1 kg increment	1.0	0.99–1.01	0.92
Use of nephrotoxic drugs	0.8	0.50–1.25	0.31
Use of ritonavir-boosted protease inhibitors	1.3	0.78–2.16	0.32
$CD4^+$ cell count per 1/μl increment	1.0	1.00–1.00	<0.001
Hypertension	2.1	1.17–3.64	0.013
Dyslipidemia	1.0	0.21–4.60	0.98
Diabetes mellitus	1.9	0.63–5.86	0.25

ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; OR, odds ratio; TDF, tenofovir disoproxil fumarate.

relative to the control continuously increased over time: at 1 year of exposure −3.8 ml/min per 1.73 m^2, at 2 years −3.6 ml/min per 1.73 m^2, at 3 years −5.5 ml/min per 1.73 m^2, at 4 years −6.6 ml/min per 1.73 m^2, and at 5 years −10.3 ml/min per 1.73 m^2 (see Table 2, Supplemental Digital Content 3, http://links.lww.com/QAD/A537, which shows adjusted loss in eGFR in the TDF group relative to the control estimated with mixed model for repeated measures). There was significant interaction between time and TDF use ($P<0.001$), suggesting that the adjusted mean loss in eGFR relative to the control increased significantly over time.

Additional analyses of renal function calculated with CKD-EPI equation also showed that TDF use doubled the risk of higher than 10 ml/min per 1.73 m^2 decrement (adjusted OR = 2.1, 95% CI 1.57–2.86, $P<0.001$) and more than 25% decrement (adjusted OR = 1.8, 95% CI 1.12–2.99, $P=0.017$). The effect of TDF use on the renal endpoint of lower than 60 ml/min per 1.73 m^2 was also marginally significant (adjusted OR = 2.7, 95% CI 0.71–10.5, $P=0.15$). The adjusted cumulative mean loss increased continuously in both the TDF and control groups: in TDF group, from −6.3 ml/min per 1.73 m^2 at 1 year to 15.0 ml/min per 1.73 m^2 at 5 years of TDF exposure, and in the control, from −4.1 ml/min per 1.73 m^2 at 1 year to −8.3 ml/min per 1.73 m^2 at 5 year of ART exposure. The cumulative mean loss in the TDF group relative to the control after 1, 2, 3, 4, and 5 years of TDF exposure was −2.2, −2.3, −3.2, −4.4, and −6.7 ml/min per 1.73 m^2, respectively, which indicated that the loss in eGFR relative to control increased over time ($P<0.001$).

Table 3. Effects of initiating tenofovir disoproxil fumarate-containing antiretroviral therapy over control on >25% decrement in estimated glomerular filtration rate relative to baseline: multivariate logistic regression analysis.

	Adjusted OR	95% CI	*P*
TDF use over control	2.1	1.50–2.90	<0.001
Baseline eGFR per 1 ml/min per 1.73 m^2	1.0	1.03–1.04	<0.001
Weight per 1 kg increment	1.0	0.98–1.01	0.37
Nephrotoxic drug use	0.7	0.47–1.03	0.073
Ritonavir-boosted protease inhibitor use	0.9	0.58–1.44	0.69
$CD4^+$ cell count per 1/μl increment	1.0	1.00–1.00	0.007
Hypertension	1.5	0.96–2.49	0.074
Dyslipidemia	0.7	0.13–3.69	0.67
Diabetes mellitus	1.8	0.77–4.30	0.17

ART, antiretroviral therapy; CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; TDF, tenofovir disoproxil fumarate.

Discussion

In this 10 years observational cohort of treatment-naive patients with low median body weight of 63 kg, initiation of TDF-containing ART doubled the risk of higher than 10 ml/min per 1.73 m^2 decrement or more than 25% decrement in eGFR relative to baseline, compared with the control patients who started ABC-containing ART, and also increased four-fold the risk of deterioration of eGFR to lower than 60 ml/min per 1.73 m^2. The effect of TDF on the decrement in eGFR was more evident in patients with body weight of lower than 70 kg (TDF use over control: adjusted OR = 2.5, 95% CI 1.55–4.00, $P<0.001$) compared with the entire study population (adjusted OR = 2.1, 95% CI 1.45–3.14, $P<0.001$), whereas the effect of TDF on renal dysfunction was only marginally significant among patients with body weight of at least 70 kg (adjusted OR = 1.7, 95% CI 0.83–3.29, $P=0.15$).

More importantly, eGFR of the patients who started TDF-containing ART decreased continuously during the 5-year observation compared with the controls who started ABC-containing ART. The adjusted mean loss relative to the control increased from −3.8 ml/min per 1.73 m^2 at 1 year of TDF exposure to −5.5 ml/min per 1.73 m^2 at 3 years, and to −10.3 ml/min per 1.73 m^2 at 5 years of TDF exposure. This decrement in eGFR associated with TDF use is alarming considering that the aging-related decrement in normal renal function is only 0.4 ml/min per year [33]. The findings of the present study warrant long-term monitoring of renal function in HIV-1-infected patients with low body weight who start TDF-containing ART.

The present study has three main strengths. First, to our knowledge, this is the first study that elucidated the effect of long-term TDF use on the prognosis of renal function among HIV-1-infected patients with low body weight. Low body weight has been identified as a risk for TDF

Table 4. Effects of initiating tenofovir disoproxil fumarate-containing antiretroviral therapy over the control on estimated glomerular filtration rate <60 ml/min per 1.73 m^2; multivariate logistic regression analysis.

	Adjusted OR	95% CI	*P*
TDF use over control	3.9	1.62–9.36	0.002
Baseline eGFR per 1 ml/min per 1.73 m^2	0.9	0.83–0.90	<0.001
Weight per 1 kg increment	1.0	0.93–1.00	0.069
Use of nephrotoxic drugs	0.6	0.22–1.52	0.27
Use of ritonavir-boosted protease inhibitors	1.4	0.47–3.89	0.57
$CD4^+$ cell count per 1/μl increment	1.0	1.00–1.00	0.94
Hypertension	1.9	0.73–5.13	0.18
Dyslipidemia	2.1	0.23–18.7	0.52
Diabetes mellitus	3.7	0.85–16.2	0.083

ART, antiretroviral therapy; CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; TDF, tenofovir disoproxil fumarate.

nephrotoxicity [16,17], and it is noteworthy that many patients with HIV-1 infection are of small body stature. Of 35.3 million estimated to be infected with HIV-1 at the end of 2012, most were from sub-Saharan Africa (25 million) and south and south-east Asia (3.9 million) [34], and studies from these regions report that HIV-1-infected patients were of low body weight (mean weight of 57.6 kg in treatment-naive patients in Zimbabwe and Uganda [35], median 60 kg in west India [36], median 56.5 kg in Thailand [18], and mean 55 kg in Vietnam [37]). Considering that body weight of these patients are even lower than that in the present study of 63 kg, the effect of long-term TDF use on renal function might be more severe among patients in these regions.

Second, the study enrolled only treatment-naive patients and followed their renal function up to 5 years after initiation of standard ART with one key drug and two NRTIs (including either TDF or ABC as control). This study design, together with its observational setting, allowed examination of the effect of long-term TDF use on the prognosis of renal function after the start of ART under 'real-world' setting, making the results of the present study more generalizable.

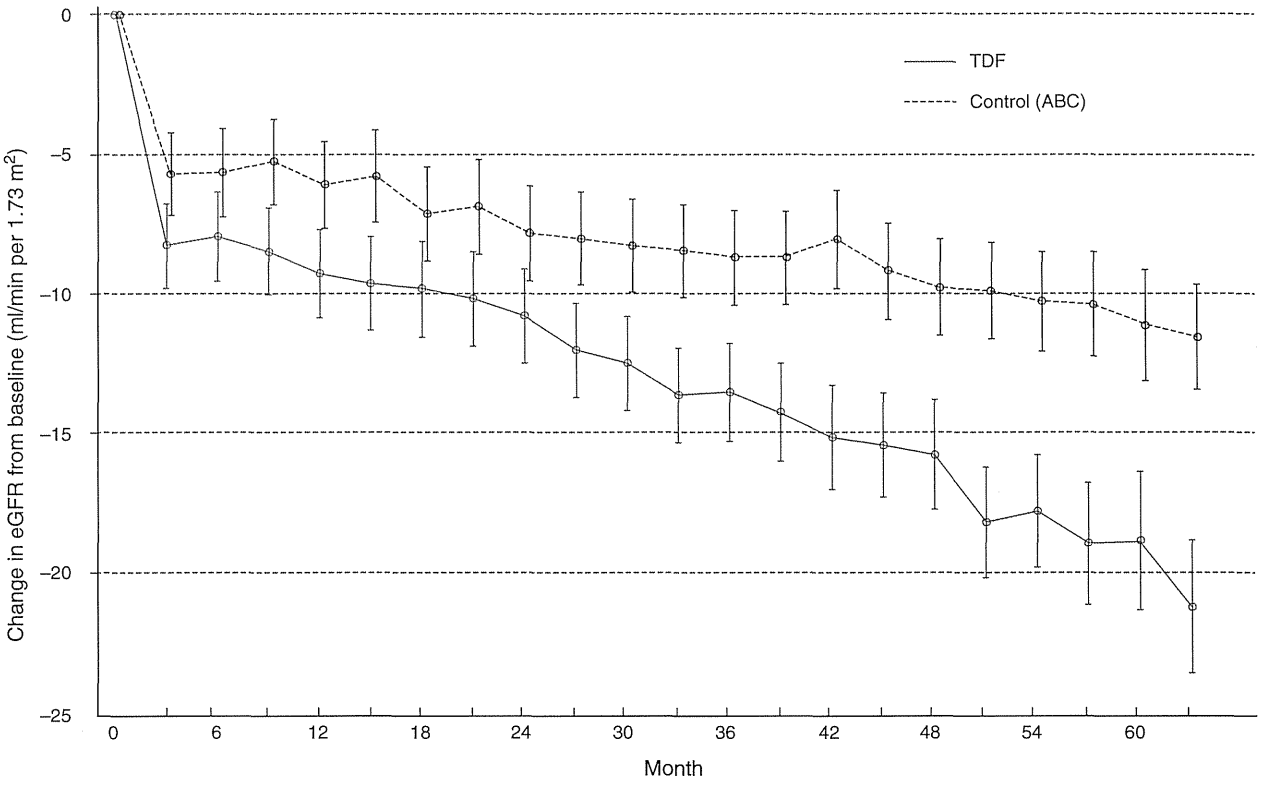

Fig. 1. Adjusted mean change in estimated glomerular filtration rate from baseline to 5 years in treatment-naive patients treated with tenofovir disoproxil fumarate-containing antiretroviral therapy (red line) and controls (patients treated with abacavir-containing ART) (black line). Least-square means and their 95% confidence intervals were estimated by the linear mixed model. The *x*-axis is labeled 'Months' to make the figure visually understandable; however, 30 days is labeled here as 1 month. Thus, 3 months equals to 90 days and so on. The model included five fixed effects (assigned treatment, baseline eGFR, baseline body weight, nephrotoxic drug use and ritonavir-boosted protease use) in this figure. ABC, abacavir; ART, antiretroviral therapy; gGFR, estimated glomerular filtration rate; TDF, tenofovir disoproxil fumarate.

Third, the study employed the Japanese equation developed by the JSN for the calculation of eGFR [23,26]. Because commonly used methods, such as MDRD and CKD-EPI equations, were validated mostly in whites and African Americans, they are probably not appropriate for people of other ethnicity or of different body stature [23,38,39]. With regard to body stature, CKD-EPI was derived from datasets of people with mean weight of 79–82 kg [24], whereas the Japanese equation was derived from the set of people with mean weight of 60.4–61 kg [23]. Accordingly, clinicians are usually encouraged to validate their own equation or use MDRD or CKD-EPI equation with ethnic coefficient [25,38]. In the present study, using the Japanese equation for eGFR for Japanese patients probably yielded a better estimate of the effect of long-term TDF use on renal function [25]. Furthermore, additional analyses with use of CKD-EPI equation adjusted with the Japanese coefficient again showed that TDF exposure increased the risk of renal dysfunction and the loss in eGFR relative to the control increased continuously up to 5 years.

Apart from the above strengths, the present study has several limitations. First, because of its observational nature, there is a potential for channeling bias by indication for TDF use. Indeed, control patients were more likely to have risks for renal dysfunction, such as diabetes mellitus, hypertension, concurrent nephrotoxic drugs, and lower $CD4^+$ cell count [16,27], than patients who started TDF-containing ART. Thus, the incidence of TDF nephrotoxicity might have been underestimated in the present study. The median observation period of the control group was longer than that of the TDF group, and this might as well contribute to underestimation of TDF nephrotoxicity. Second, a high percentage of our study population used PI/r, which is considered a risk for TDF nephrotoxicity [28]. Although it is difficult to completely exclude the effect of concurrent PI/r, it should be noted that PI/r use itself (even without concurrent TDF) has been considered a risk for CKD [30,40], and the percentage of PI/r use was similarly high in both the TDF and control group, suggesting that PI/r affected renal function of the control patients to some extent as well. Furthermore, the use of PI/rs did not correlate with any of the three renal outcomes in this study (Tables 2–4). Third, all study participants were Japanese and we had a small number of women. Further studies are needed to determine whether the findings of this study are also applicable to women and patients of different racial background.

In conclusion, this long-term observational study of HIV-1-infected patients with predominantly low body weight demonstrated that initiation of TDF-containing ART doubled the risk of higher than 10 ml/min per 1.73 m^2 decrement and more than 25% decrement in eGFR, and also four-fold increased the risk of deterioration of eGFR to lower than 60 ml/min per 1.73 m^2, compared with the controls who started ABC-containing ART. The loss in eGFR in the TDF group relative to the control increased continuously over time and reached −10 ml/min per 1.73 m^2 at 5 years of TDF exposure. The results of the study certainly warrant regular and long-term monitoring of renal function in patients with low body weight who start TDF-containing ART. Further larger studies are needed to confirm the long-term renal prognosis with TDF use in patients with low body weight.

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Conflicts of interest

S.O. has received honoraria and research grants from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Pfizer, Co., and Roche Diagnostics K.K.; has received honoraria from Astellas Pharmaceutical K.K., Bristol-Myers K.K., Daiichisankyo, Co., Dainippon Sumitomo Pharma, Co., GlaxoSmithKline, K.K., Taisho Toyama Pharmaceutical, Co., Torii Pharmaceutical, Co., and ViiV Healthcare. H.G. has received honoraria from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Torii Pharmaceutical, Co., Roche Diagnostics K.K., and ViiV Healthcare, Co.

The remaining authors declare no conflict of interest.

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Diagnostic Utility of Quantitative Plasma Cytomegalovirus DNA PCR for Cytomegalovirus End-Organ Diseases in Patients With HIV-1 Infection

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Objective: To investigate the diagnostic value of quantitative plasma cytomegalovirus (CMV)-DNA polymerase chain reaction (PCR) for CMV end-organ diseases (CMV-EOD) in patients with HIV-1 infection.

Design: Single-center cross-sectional study.

Methods: The study subjects were HIV-1–infected patients with CD4 ≤200 per microliter, who had undergone ophthalmologic examination with plasma CMV-DNA PCR measured within 7 days. CMV retinitis and other CMV-EOD were diagnosed according to the ACTG criteria. PCR value was converted into the WHO international standard.

Results: CMV retinitis and all CMV-EOD were diagnosed in 23 (5%) and 37 (8%) of the 461 study patients, respectively. CMV-DNA was undetectable (<185 IU/mL) in 2 patients with CMV retinitis and 1 with encephalitis. The area under the receiver operating characteristic curve of CMV-DNA for CMV retinitis and all CMV-EOD were 0.80 [95% confidence interval (CI): 0.71 to 0.89] and 0.82 (0.75 to 0.89), respectively. The sensitivity, specificity, positive predictive value, and negative predictive value for each cutoff value of CMV-DNA were as follows: for CMV retinitis, ≥10,086 IU/mL: 26.1%, 94.1%, 18.8%, 96%; ≥2946 IU/mL; 56.5%, 86.8%, 18.3%, 97.4%; ≥959 IU/mL; 60.9%, 78.1%, 12.7%, 97.4%; detectable CMV-DNA (≥185 IU/mL): 91.3%, 48.2%, 8.5%, 99.1%; for all CMV-EOD: ≥10,086 IU/mL: 32.4%, 95.3%, 37.5%, 94.2%; ≥2946 IU/mL; 54.1%, 88%, 28.2%, 95.6%; ≥959 IU/mL; 62.2%, 79.5%, 20.9%, 96%; detectable CMV-DNA; 91.9%, 49.5%, 13.7%, 98.6%.

Conclusions: Plasma CMV-DNA PCR has a high diagnostic value for both CMV retinitis and all CMV-EOD in patients with advanced HIV-1 infection. A cutoff value of CMV-DNA ≥10,086 IU/mL and ≥2946 IU/mL yields high specificity, whereas undetectable CMV-DNA load (<185 IU/mL) likely rules out CMV-EOD.

Key Words: cytomegalovirus infection, CMV-DNA PCR, HIV-1 infection, CMV retinitis, CMV end-organ diseases

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INTRODUCTION

Although antiretroviral therapy (ART) has substantially improved the prognosis of patients with HIV-1 infection, a large number of patients are still diagnosed with HIV-1 infection at a late stage, often with concurrent opportunistic infections.[1,2] Cytomegalovirus end-organ disease (CMV-EOD) is a major debilitating opportunistic infection in patients with advanced HIV-1 infection.[3,4] Among CMV end-organ diseases, retinitis is the most common clinical manifestation, which can cause total blindness.[5] Other manifestations include colitis, pneumonitis, esophagitis, and various neurological diseases.[3,6] Although the wide availability of ART has substantially reduced the incidence of CMV-EOD,[7] CMV-EOD is associated with increased mortality even in the ART era.[8]

In HIV-1–infected patients, blood tests to detect CMV by polymerase chain reaction (PCR) is not recommended for the diagnosis of CMV-EOD by the American Adult and Adolescent Opportunistic Infection Guidelines,[3] in contrast with the management of solid-organ transplantation where real-time quantitative PCR is the standard of care for the diagnosis of CMV-EOD.[9,10] One major problem related to the assessment of the diagnostic utility of quantitative PCR for CMV-EOD is that there is often poor interinstitutional correlation of quantitative PCR tests,[11] which curtails the establishment of cutoff values for clinical decision-making. In this regard, the WHO International Standard, which attempts to establish reproducibility in quantitative CMV load across laboratories, has only become available in 2010.[12,13] Hence, only a few studies in the field of HIV-1 infection have investigated this issue.[14–17] Another issue in diagnosis of CMV-EOD is that definitive diagnosis of CMV colitis is sometimes difficult in a small number of patients because tissue biopsy can only be obtained through colonoscopy. The latter is not always feasible, especially in patients with poor general condition, thus resulting in possible underdiagnosis of

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CMV-EOD. However, diagnosis of CMV retinitis is relatively easy with ophthalmologic examination including dilated retinal examination using indirect ophthalmoscopy by experienced ophthalmologist.

This study was designed to assess the diagnostic value of quantitative plasma CMV-DNA PCR for CMV-EOD in patients with advanced HIV-1 infection, with a special effort to tackle the abovementioned 2 obstacles by (1) using a PCR method that is traceable to the WHO international standard and (2) only enrolling patient who underwent ophthalmologic examination to avoid underdiagnosis of CMV retinitis, the most prevalent CMV-EOD, and calculating the diagnostic utilities of CMV PCR separately for CMV retinitis and all CMV-EOD.

METHODS

Study Design, Setting, and Participants

We conducted a single-center cross-sectional study to investigate the usefulness of quantitative plasma CMV-DNA PCR for the diagnosis of CMV-EOD among patients with advanced HIV-1 infection at AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo, Japan. This center is the largest referral center for HIV-1 infection in Japan.[18] The following criteria were applied for enrollment: inclusion criteria—(1) HIV-1–infected patients aged ≥18 years who first visited our clinic between January 2004 and December 2013 and underwent full ophthalmologic examination, (2) patients with CD4 count ≤200 per microliter, and (3) plasma CMV-DNA PCR was measured within 7 days from the day of the ophthalmologic examination; and exclusion criterion—patients who had already been diagnosed with CMV retinitis or other CMV-EOD at the time of referral to our clinic, because it is often difficult to confirm retinal photography or pathology, which are required for the diagnosis of CMV-EOD according to the standard ACTG criteria for such cases.[19] At our clinic, ophthalmologic examination including dilated retinal examination using indirect ophthalmoscopy by experienced ophthalmologist is routinely conducted on first visit to our clinic. When the diagnosis of CMV retinitis was uncertain, the examination was repeated within 1–4 weeks and then confirmed by at least 2 ophthalmologists. For patients with suspected CMV encephalitis, CMV-DNA PCR of cerebrospinal fluid was routinely assessed, and gastroscopy or colonoscopy was performed with biopsy for those with suspected CMV esophagitis/colitis. Plasma CMV-DNA PCR was also routinely conducted for HIV-1-infected patients with CD4 ≤200 per microliter.

The study was approved by the Human Research Ethics Committee of NCGM. All patients included in this study provided written informed consent for their clinical and laboratory data to be used and published for research purposes. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Measurements

The results of the first ophthalmologic examination for each patient were extracted from the medical charts, together with plasma CMV-DNA PCR value determined within 7 days of the examination. The diagnosis of CMV retinitis was based on the standard ACTG criteria of "confirmed CMV retinitis," which include diagnosis by an experienced ophthalmologist and documentation of CMV retinitis by retinal photography.[19] Data on other CMV-EOD were also extracted from the medical records; the diagnosis of other CMV-EOD was based on the standardized ACTG criteria and confirmed within 4 weeks of ophthalmologic examination.[19] Baseline characteristics [age, sex, ethnicity, history of AIDS, route of HIV-1 transmission, and treatment status for HIV-1 infection (either treatment naive or experienced)], CD4 count, and HIV-1 viral load were also collected. For CD4 count and HIV-1 viral load, the data closest to and preceding the day of the first ophthalmologic examination were used. Systemic steroid use, anti-CMV treatment, and chemotherapy were also recorded. They were defined as therapies administered either orally or intravenously within 1 month preceding the ophthalmologic examination.

Measurement of Quantitative CMV-DNA PCR

Throughout the study period, CMV PCR tests were conducted within 24 hours after sample blood collection using the geniQ CMV Real-Time PCR assay. The tests were performed at KITASATO-OTSUKA Biomedical Assay Laboratories Co. (KOBAL; Sagamihara, Japan), which is accredited by ISO15189. Details of the procedures, probes, and primers for the assay were reported previously[20] and run on the ABI 7900HT system (Applied Biosystems, Foster City, CA). The assay used had a CMV-DNA limit of detection of 200 copies per milliliter. The geniQ CMV correlates well with COBAS AmpliPrep/COBAS TaqMan CMV Test (Roche Molecular System, Branchburg, NJ); [R^2 = 0.9763, y = 0.9784x + 0.0427, where y = $\log_{10}$(geniQ CMV copies/mL) and x = $\log_{10}$(COBAS AmpliPrep/COBAS TaqMan CMV Test), n = 59, unpublished data]. Because COBAS AmpliPrep/COBAS TaqMan CMV Test is traceable to the WHO First International Standard with coefficient of 1.1,[12] the geniQ CMV was converted to WHO international unit (IU) using following formula: = 0.91 × 10 raised to the power of {[$\log_{10}$(geniQ CMV copies/mL) − 0.0427]/0.9784}.

Statistical Analysis

Baseline characteristics were compared between patients with and without CMV retinitis, and those with and without CMV-EOD, using the Student *t* test and χ^2 test (Fisher exact test) for continuous and categorical variables. The univariate logistic regression model was applied to estimate the effects of different CMV-DNA cutoff values [CMV-DNA PCR ≥10,000 copies/mL (10,086 IU/mL), ≥3000 copies/mL (2946 IU/mL), and ≥1000 copies/mL (959 IU/mL), and detectable CMV load [≥200 copies/mL (185 IU/mL)] and other variables on the occurrence of CMV retinitis and all CMV-EOD. Undetectable CMV-DNA [<200 copies/mL (185 IU/mL)] was treated as 10 IU/mL (1 $\log_{10}$ IU/mL) in logarithmic calculations. Multivariate logistic regression model was applied to estimate the effects of each CMV-DNA cutoff values on the development of CMV retinitis and all CMV-EOD. The model for CMV retinitis was adjusted for age and CD4 count, because low CD4

count is an established risk factor for CMV retinitis[21] and also for variables with P value of <0.05 in univariate analysis (other CMV diseases). The model for all CMV-EOD was adjusted for age, CD4 count, and anti-CMV treatment. Sex was not added to the models because all patients with retinitis and all except 1 CMV-EOD patient were males. Receiver operating characteristic (ROC) curves were constructed, and the area under the curve (AUC) was estimated with 95% confidence interval (CI) to quantify the accuracy of CMV-DNA PCR. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and adjusted odds ratio for the diagnosis of CMV retinitis and all CMV-EOD were calculated for abovementioned different cutoff values. Additional analyses were conducted for data of patients with CD4 count of ≤ 100 and those with CD4 count of ≤ 50 per microliter. Statistical significance was defined as 2-sided P values <0.05. We used odds ratios (ORs) with 95% CIs. All statistical analyses were performed with the Statistical Package for Social Sciences version 21.0 (SPSS, Chicago, IL).

RESULTS

As shown in Figure 1, 1635 patients visited our clinic for the first time during the study period and underwent ophthalmologic examination. Of the 764 patients who had CD4 <200 per microliter, 461 (60%) patients underwent CMV-DNA PCR within 7 days of the examination and were included as the study patients. They were mostly Asian males and treatment naive for HIV-1 infection (Table 1). The median CD4 count and HIV-1 load were 42 per microliter [interquartile range (IQR), 18–78/μL] and 5.23 $\log_{10}$copies per milliliter (IQR, 4.85–5.68 $\log_{10}$copies/mL), respectively. CMV-DNA was detected in 248 (54%), 218 (58%), and 164 (62%) patients of the total study population (CD4 ≤ 200/μL), patients with CD4 ≤ 100 per microliter, and those with CD4 ≤ 50 per microliter, respectively.

CMV retinitis was diagnosed in 23 (5.0%) patients. Furthermore, 8 CMV colitis, 5 encephalitis, and 4 esophagitis cases were diagnosed. All encephalitis cases had documented detection of viral nucleic acids in cerebrospinal fluid, and all colitis and esophagitis had documented pathological evidence of CMV infection.[19] Because 3 patients had more than 1 CMV-EOD, 37 (8.0%) patients were diagnosed with CMV-EOD. The median CD4 counts of patients with CMV retinitis and those with any CMV-EOD were 31/μL (IQR, 16-74/μL; range, 7–158/μL) and 25/μL (IQR, 10–57/μL; range, 3–158/μL), respectively (Table 1).

Patients with CMV retinitis or CMV-EOD had higher CMV load and were more likely to have CMV load of $\geq 10{,}086$ IU/mL, ≥ 2946 IU/mL, ≥ 959 IU/mL, and detectable load than patients without retinitis or CMV-EOD, respectively (Table 1). CMV-DNA was undetectable in 2 patients with retinitis and 1 patient with encephalitis. None of these 3 patients had received anti-CMV treatment within 1 month preceding the day of PCR examination. Patients with CMV retinitis and those with CMV-EOD tended to be on anti-CMV treatment compared with those free of these diseases ($P = 0.095$ and $P = 0.018$, respectively). There was no difference in CD4 count between patients with CMV retinitis and without retinitis, whereas CD4 count of the patients with CMV-EOD was marginally lower than that of those free of CMV-EOD ($P = 0.053$). There was no difference

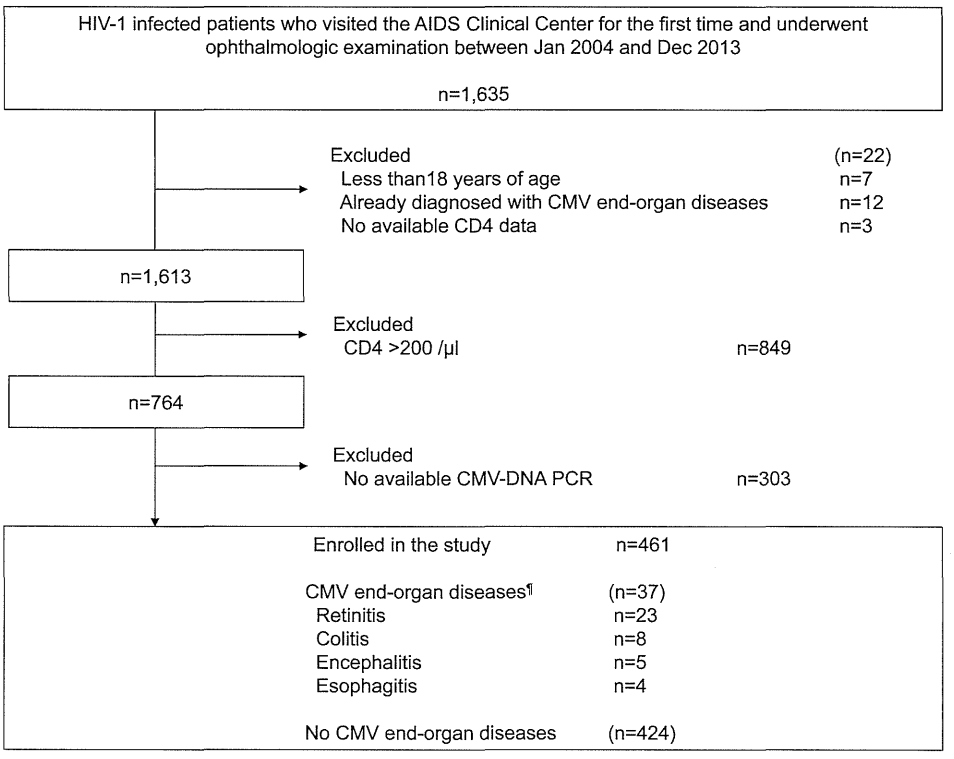

FIGURE 1. Patient enrollment process. ¶Three patients had more than 1 CMV-EOD.

TABLE 1. Baseline Characteristics of HIV-1-Infected Patients With and Without Cytomegalovirus Retinitis and CMV-EOD

		CMV Retinitis			All CMV-EOD		
	All Study Patients (n = 461)	CMV Retinitis (n = 23)	No CMV Retinitis (n = 438)	*P*	All CMV-EOD (n = 37)	No CMV-EOD (n = 424)	*P*
Sex (male), n (%)	433 (94)	23 (100)	410 (94)	0.39	36 (97)	397 (94)	0.72
Age*	39 (33–48)	42 (33–53)	39 (33–47)	0.23	41 (34–50)	39 (33–47)	0.24
CMV load (log_{10} IU/mL)*	2.27 (1.00–2.91)	3.47 (2.67–4.00)	2.27 (1.00–2.82)	<0.001	3.47 (2.67–4.31)	2.27 (1.00–2.75)	<0.001
≥10,086 IU/mL, n (%)	32 (7)	6 (26)	26 (6)	0.003	12 (32)	20 (5)	<0.001
≥2946 IU/mL, n (%)	71 (15)	13 (57)	58 (13)	<0.001	20 (54)	51 (12)	<0.001
≥959 IU/mL, n (%)	110 (24)	14 (61)	96 (22)	<0.001	23 (62)	87 (21)	<0.001
Detectable (≥185 IU/mL), n (%)	248 (54)	21 (91)	227 (52)	<0.001	34 (92)	214 (51)	<0.001
CMV diseases other than retinitis, n (%)	17 (4)	3 (13)	14 (3)	0.047	NA	NA	NA
CD4 count (/μL)*	42 (18–78)	31 (16–74)	43 (18–78)	0.55	25 (10–57)	43 (19–81)	0.053
HIV RNA viral load (log_{10} copies/mL)*	5.23 (4.85–5.68)	5.32 (4.57–5.84)	5.23 (4.85–5.67)	0.52	5.51 (4.89–5.72)	5.20 (4.84–5.67)	0.89
ART, n (%)	40 (9)	3 (13)	37 (8)	0.44	3 (8)	37 (9)	1.00
Systemic steroid use, n (%)	134 (29)	5 (22)	129 (30)	0.49	13 (35)	121 (29)	0.45
Anti-CMV treatment, n (%)	36 (8)	4 (17)	32 (7)	0.095	7 (19)	29 (7)	0.018
Chemotherapy, n (%)	10 (2)	1 (4)	9 (2)	0.40	1 (3)	9 (2)	0.57
History of AIDS, n (%)	339 (74)	23 (100)	316 (72)	0.001	37 (100)	302 (71)	<0.001
Homosexual contact, n (%)	365 (79)	17 (74)	348 (80)	0.60	29 (78)	336 (79)	0.84
Diabetes mellitus, n (%)	15 (3)	1 (4)	14 (3)	0.54	2 (5)	13 (3)	0.34

*Median (IQR).
NA, not applicable.

in age, HIV RNA viral load, and the percentage of patients on ART, on systemic steroid use, on chemotherapy, and with diabetes mellitus, between patients with and without CMV retinitis and patients with and without CMV-EOD (Table 1).

Univariate analysis showed that CMV-DNA ≥10,086, ≥2946, and ≥959 IU/mL were all associated with CMV retinitis, whereas undetectable load (<185 IU/mL) was inversely associated with CMV retinitis (OR, 0.1; 95% CI: 0.02 to 0.44; $P = 0.002$) (Table 2). Concurrent CMV diseases other than retinitis were also associated with retinitis and on anti-CMV therapy were marginally associated with retinitis. Similarly, CMV-DNA ≥10,086, ≥2946, and ≥959 IU/mL were all associated with CMV-EOD, whereas undetectable CMV load was inversely associated with CMV-EOD (OR, 0.1; 95% CI: 0.03 to 0.30; $P < 0.001$). The use of anti-CMV treatment was also associated with CMV-EOD.

ROC-AUC of CMV-DNA PCR was 0.80 (95% CI: 0.71 to 0.89) for CMV retinitis and 0.82 (95% CI: 0.75 to 0.89) for all CMV-EOD. The sensitivity, specificity, PPV, NPV, and the result of multivariate analysis for each cutoff value of CMV-DNA for the diagnosis of CMV retinitis are shown in (Table 3). CMV-DNA PCR of ≥10,086 and of ≥2946 IU/mL had 94.1% and 86.8% specificity, respectively, whereas detectable PCR had 91.3% sensitivity (NPV 99.1%). For all CMV-EOD, CMV-DNA of ≥10,086 and of ≥2946 IU/mL had 95.3% and 88% specificity, respectively, whereas detectable PCR had 91.9% sensitivity (NPV 98.6%) (Table 4).

Among patients with CD4 count of ≤100/μL (n = 376) and ≤50/μL (n = 265), 20 (5.3%) and 14 (5.3%) patients, respectively, were diagnosed with CMV retinitis and 33 (8.8%) and 26 (9.8%) patients, respectively, were diagnosed with any CMV-EOD. For patients with CD4 ≤100/μL, ROC-AUC of the CMV-DNA PCR for the diagnosis of CMV retinitis was 0.77 (95% CI: 0.67 to 0.87), and for the diagnosis of all CMV-EOD, it was 0.79 (95% CI: 0.71 to 0.87). For those with CD4 ≤50/μL, ROC-AUC for CMV retinitis was 0.73 (95% CI: 0.62 to 0.84) and for CMV-EOD, it was 0.76 (95% CI: 0.67 to 0.85).

The sensitivity, specificity, PPV, and NPV for each cutoff value for the diagnosis of CMV retinitis in patient with CD4 counts ≤100/μL and ≤50/μL are shown in (Table 3). For patients with CD4 ≤100/μL, CMV-DNA PCR of ≥10,086 and ≥2946 IU/mL yielded 93.3% and 84.8% specificity, respectively, whereas detectable CMV-DNA had 90% sensitivity (NPV 98.7%). Similarly, for patients with CD4 ≤50/μL, CMV-DNA of ≥10,086 and ≥2946 IU/mL had 92% and 82.1% specificity, respectively, and detectable CMV-DNA had 92.9% sensitivity (NPV 99%). These parameters for the diagnosis of all CMV-EOD in patients with CD4 count ≤100/μL and ≤50/μL are shown in (Table 4). For patients with CD4 ≤100/μL, CMV-DNA PCR of ≥10,086 and ≥2946 IU/mL had 94.5% and 86% specificity, respectively, whereas detectable CMV-DNA had 90.9% sensitivity (NPV 98.1%). Similarly, for patients with CD4 ≤50/μL, CMV-DNA of ≥10,086 and ≥2946 IU/mL had 93.7% and 83.7% specificity, respectively, and detectable CMV-DNA had 92.3% sensitivity (NPV 98%).

DISCUSSION

This cross-sectional study showed that quantitative plasma CMV-DNA PCR test is a useful surrogate marker for the diagnosis of both retinitis and all CMV-EOD in

TABLE 2. Results of Univariate Analysis to Estimate the Association of Each Variable With CMV Retinitis and All CMV-EOD

	CMV Retinitis			All CMV-EOD		
	OR	95% CI	*P*	OR	95% CI	*P*
CMV load ≥10,086 IU/mL	5.6	2.03 to 15.4	0.001	9.7	4.26 to 22.1	<0.001
CMV load ≥2946 IU/mL	8.5	3.57 to 20.3	<0.001	8.6	4.23 to 17.5	<0.001
CMV load ≥959 IU/mL	5.5	2.33 to 13.2	<0.001	6.4	3.15 to 12.9	<0.001
Undetectable CMV load (<185 IU/mL)	0.1	0.02 to 0.44	0.002	0.1	0.03 to 0.30	<0.001
Age per 1 yr increment	1.0	0.99 to 1.06	0.24	1.0	0.99 to 1.05	0.24
CD4 per 1/μL increment	1.0	0.99 to 1.01	0.55	1.0	0.98 to 1.00	0.057
HIV-1 RNA load per 1 $\log_{10}$ copies/mL increment	0.9	0.57 to 1.32	0.52	1.0	0.71 to 1.49	0.89
ART	1.6	0.46 to 5.73	0.45	0.9	0.27 to 3.15	0.90
CMV diseases other than retinitis	4.5	1.21 to 17.1	0.025	NA	NA	NA
Anti-CMV treatment	2.7	0.86 to 8.32	0.090	3.2	1.29 to 7.86	0.012
Chemotherapy	2.2	0.26 to 17.9	0.47	1.3	0.16 to 10.4	0.82
Systemic steroid use	0.7	0.24 to 1.83	0.43	1.4	0.67 to 2.75	0.40

HIV-1–infected patients with CD4 count ≤200/μL. The cutoff value of ≥10,086 IU/mL yielded 94.1% specificity for CMV retinitis and 95.3% specificity for all CMV-EOD, and the cutoff of ≥2946 IU/mL had 86.8% specificity for retinitis and 88% specificity for all CMV-EOD. Undetectable load (<185 IU/mL) can likely rule out CMV retinitis and EOD, since undetectable load showed 91.3% and 91.9% sensitivity (99.1% and 98.6% NPV) for retinitis and all EOD, respectively. In subgroup analysis of patients with CD4 count of ≤100/μL and ≤50/μL, the results were also similar. Especially, the result that undetectable CMV load can rule out any CMV-EOD with >90% sensitivity (NPV >98%) should help clinical decision making as a surrogate marker.

This study has 2 major strengths. First, to the best of our knowledge, this is the first study that has investigated the diagnostic value of quantitative CMV-DNA PCR for CMV-EOD in patients with HIV-1 infection, with the results converted to the WHO international unit to allow comparison of the cutoff values with those obtained by other laboratories. Another important dimension of the study was the processing of the PCR test within 24 hours after blood sample collection. In this regard, the stability of CMV viral load in blood and plasma samples stored over a long period of time has not been well validated.[22,23]

Second, we only included patients who underwent full ophthalmologic examination to avoid underdiagnosis of retinitis to appropriately evaluate the diagnostic value of plasma CMV-DNA PCR. For other CMV diseases, such as esophagitis and colitis, underdiagnosis is possible to some extent because in clinical practice, not all patients with difficulty in swallowing or abdominal pain can undergo endoscopy and pathological examination, which are required for the diagnosis of CMV gastrointestinal diseases.[24,25] However, it is relatively easy for experienced ophthalmologists to make a definitive diagnosis for CMV retinitis. Furthermore, CMV retinitis forms the largest proportion of

TABLE 3. Diagnostic Accuracy of CMV-DNA PCR for CMV Retinitis Using Different Cutoff Values for the Entire Study Population (CD4 ≤200/μL), patients With CD4 ≤100/μL, and those With ≤50/μL

					Adjusted OR	
	Sensitivity, %	Specificity, %	PPV, %	NPV, %	OR (95% CI)	*P*
Study patients (N = 461)						
CMV-DNA PCR ≥10,086 IU/mL	26.1	94.1	18.8	96.0	4.2 (1.39 to 12.9)	0.011
CMV-DNA PCR ≥2946 IU/mL	56.5	86.8	18.3	97.4	7.9 (3.12 to 20.1)	<0.001
CMV-DNA PCR ≥959 IU/mL	60.9	78.1	12.7	97.4	5.0 (2.02 to 12.6)	0.001
Detectable CMV-DNA PCR (≥185 IU/mL)	91.3	48.2	8.5	99.1	9.0 (2.02 to 40.0)	0.004
Patients with CD4 ≤100 (n = 376)						
CMV-DNA PCR ≥10,086 IU/mL	20	93.3	14.3	95.4	2.8 (0.75 to 10.2)	0.13
CMV-DNA PCR ≥2946 IU/mL	55	84.8	16.9	97.1	6.7 (2.53 to 18.0)	<0.001
CMV-DNA PCR ≥959 IU/mL	60	75.3	12	97.1	4.3 (1.65 to 11.4)	0.003
Detectable CMV-DNA PCR (≥185 IU/mL)	90	43.8	8.3	98.7	6.6 (1.48 to 29.7)	0.013
Patients with CD4 ≤50 (n = 265)						
CMV-DNA PCR ≥10,086 IU/mL	21.4	92	13	95.5	2.3 (0.49 to 11.0)	0.29
CMV-DNA PCR ≥2946 IU/mL	42.9	82.1	11.8	96.3	2.9 (0.90 to 9.37)	0.075
CMV-DNA PCR ≥959 IU/mL	50	72.5	9.2	96.3	2.4 (0.76 to 7.43)	0.14
Detectable CMV-DNA PCR (≥185 IU/mL)	92.9	39.8	7.9	99	7.5 (0.95 to 58.8)	0.057

TABLE 4. Diagnostic Accuracy of CMV-DNA PCR for All CMV Organ Diseases Using Different Cutoff Values for the Entire Study Population (CD4 ≤200/μL), Patients With CD4 ≤100/μL, and Those With ≤50/μL

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Adjusted OR OR (95% CI)	P
Study patients (n = 461)						
CMV-DNA PCR ≥10,086 IU/mL	32.4	95.3	37.5	94.2	7.5 (3.16 to 18.0)	<0.001
CMV-DNA PCR ≥2946 IU/mL	54.1	88	28.2	95.6	7.2 (3.47 to 15.2)	<0.001
CMV-DNA PCR ≥959 IU/mL	62.2	79.5	20.9	96	5.4 (2.59 to 11.1)	<0.001
Detectable CMV-DNA PCR (≥185 IU/mL)	91.9	49.5	13.7	98.6	9.7 (2.91 to 32.5)	<0.001
Patients with CD4 ≤100 (n = 376)						
CMV-DNA PCR ≥10,086 IU/mL	27.3	94.5	32.1	93.1	5.1 (1.95 to 13.3)	0.001
CMV-DNA PCR ≥2946 IU/mL	51.5	86	26.2	94.9	5.8 (2.67 to 12.5)	<0.001
CMV-DNA PCR ≥959 IU/mL	60.6	76.7	20	95.3	4.5 (2.09 to 9.56)	<0.001
Detectable CMV-DNA PCR (≥185 IU/mL)	90.9	45.2	13.8	98.1	7.6 (2.27 to 25.7)	0.001
Patients with CD4 ≤50 (n = 265)						
CMV-DNA PCR ≥10,086 IU/mL	30.8	93.7	34.8	92.6	5.8 (1.95 to 17.4)	0.002
CMV-DNA PCR ≥2946 IU/mL	46.2	83.7	23.5	93.5	3.8 (1.58 to 9.08)	0.003
CMV-DNA PCR ≥959 IU/mL	53.8	74.1	18.4	93.7	3.0 (1.27 to 6.99)	0.012
Detectable CMV-DNA PCR (≥185 IU/mL)	92.3	41.4	14.6	98	7.9 (1.81 to 34.3)	0.006

CMV-EOD in patients with HIV-1 infection.[3,6] These were the reasons for setting up the abovementioned inclusion criteria for study patients and for separately analyzing the diagnostic value of CMV load for CMV retinitis and all CMV-EOD. The results for CMV retinitis and all CMV-EOD were very similar.

Our results that quantitative plasma CMV-DNA PCR test is useful for the diagnosis of CMV-EOD, especially in ruling out CMV-EOD, are in conflict with the American Adult and Adolescent Opportunistic Infection Guidelines, which do not recommend the use of blood tests to detect CMV by PCR for the diagnosis of CMV-EOD.[3] However, it needs to be noted that, to the best of our knowledge, only 3 studies have previously investigated the utility of blood CMV load for the diagnosis of CMV-EOD with cross-sectional study design,[14–16] although many other studies either longitudinally investigated the utility of CMV load for the prediction or progression of CMV-EOD or death during the follow-up period,[26–34] or examined the effectiveness of preemptive therapy for CMV-EOD based on positive CMV load.[19,35–38] The major limitations of the abovementioned 3 studies that investigated the diagnostic utility of blood CMV load for CMV-EOD included small sample size (n = 70 for Yoshida et al,[14] n = 58 for Pellegrin et al,[16] and n = 53 for Brantsaeter et al[15]), and importantly, their results were not convertible to the WHO international unit. In comparison, this study included a far larger number of study population of 461 patients, and the results were convertible to the WHO international unit. These 2 features probably explain the reasons why the results of this study are in conflict with the recommendations made by the abovementioned American Guidelines.[3]

Apart from the abovementioned strengths of this study, we need to acknowledge some study limitations. First, although all study patients underwent full ophthalmologic examination for the screening of CMV retinitis, due to the nature of observational study, not all patients necessarily underwent the appropriate procedures (eg, endoscopy or biopsy) required to establish the diagnosis of CMV-EOD. Thus, underdiagnosis of CMV-EOD other than retinitis is possible in this study. However, as explained above, the majority of CMD-EOD cases had retinitis (23 of 37), consistent with previous studies,[3,6] and the diagnostic parameters for each cutoff value of CMV load was similar for CMV retinitis and all CMV-EOD, suggesting that substantial underdiagnosis of CMV-EOD other than retinitis was unlikely. Second, this study did not exclude patients who have received anti-CMV treatment, although the treatment could have affected the value of CMV-DNA PCR. This is because it is not sometimes easy to judge whether the patient had received anti-CMV treatment or not, and the number of patients who received either oral or intravenous anti-CMV treatment within 1 month preceding the ophthalmologic examination was relatively small; 36 (8%) of all study patients, 4 (17%) of patients with CMV retinitis, and 7 (19%) of those with any CMV-EOD. Furthermore, exclusion of patients with anti-CMV treatment did not alter the results; the cutoff value of ≥10,086 IU/mL yielded 95.3% and 96.2% specificity for CMV retinitis and for all CMV-EOD, respectively, and undetectable load had 89.5% and 90% sensitivity (99% and 98.5 NPV) for retinitis and all EOD, respectively.

In conclusion, quantitative plasma CMV-DNA PCR using WHO international unit was a useful surrogate diagnostic marker for CMV-EOD in HIV-1–infected patients with CD4 count ≤200/μL. The cutoff value of ≥10,086 IU/mL yielded 94.1% specificity for retinitis and 95.3% specificity for all CMV-EOD, whereas undetectable load (<185 IU/mL) had 91.3% and 91.9% sensitivity (99.1% and 98.6% NPV) for retinitis and all CMV-EOD, respectively. Especially, the result that undetectable load could rule out any CMV-EOD with >90% sensitivity (>98% NPV) can be helpful in clinical practice for the screening of CMV-EOD in patients with HIV-1 infection.