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Introduction

The fixed-dose combinations of tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg and abacavir sulfate 600 mg/lamivudine 300 mg are components of antiretroviral therapy for treatment-naïve patients with HIV-1 infection in developed countries (1, 2). The efficacy and safety of tenofovir/emtricitabine (TDF/FTC) and abacavir/lamivudine (ABC/3TC) remain the focus of ongoing debate. The ACTG 5202 trial demonstrated that the viral efficacy of ABC/3TC is inferior to that of TDF/FTC among treatment-naïve patients with a baseline HIV viral load of >100,000 copies/mL receiving efavirenz or ritonavir-boosted atazanavir as a key drug (3). On the other hand, the HEAT study showed that the viral efficacy of ABC/3TC is not inferior to that of TDF/FTC, regardless of the baseline viral load when used in combination with lopinavir/ritonavir (4).

With regard to safety, the occurrence of ABC-associated serious hypersensitivity reactions, the most important adverse effect of ABC affecting 5-8% of patients, has limited its use (5). However, screening for HLA-B*5701 or prescribing ABC in HLA-B*5701-negative populations, such as the Japanese, can reduce the incidence of immunologically-confirmed hypersensitivity to 0% (6, 7). Another negative aspect of ABC use is its association with myocardial infarction, as reported by the D:A:D study (8). However, the possible association of myocardial infarction with ABC was not confirmed by a recent meta-analysis report of the US Food and Drug Administration (9). On the other hand, renal proximal tubular damage leading to renal dysfunction and a loss of phosphate, which can result in decreased bone mineral density, is a well-known adverse effect of TDF (10-14).

Taking this background into account, the American Department of Health and Human Services (DHHS) Guidelines place TDF/FTC as the preferred drug and ABC/3TC as an alternative choice, whereas other international guidelines, including the European AIDS Clinical Society (EACS) Guidelines and the Japanese Guidelines, recommend both TDF/FTC and ABC/3TC as preferred choices (1, 2, 15).

Randomized control trials comparing TDF/FTC and ABC/3TC have been conducted in the US and Europe, but not in other parts of the world (4, 16, 17). The efficacy and safety of these two fixed-dose regimens in patients with different genetic backgrounds and body statures might not be similar to the results of previous trials, especially considering that the prevalence of HLA-B*5701 is zero in the Japanese population (7). Moreover, the degree of decrement in the renal function with TDF is larger in patients with a low body weight, such as the Japanese, which might limit the use of TDF in patients with a high risk for renal dysfunction (18-20).

Based on the above described background, the present randomized trial was originally designed in 2007 to elucidate whether the viral efficacy of ABC/3TC is not inferior to that of TDF/FTC with ritonavir-(100 mg) boosted atazanavir (300 mg) in treatment-naïve Japanese patients, whose body weight is much lower than Whites or Blacks (21). However, the independent data and safety monitoring board (DSMB) recommended that the protocol be modified to examine the efficacy, safety and tolerability among Japanese patients with HIV-1 infection for 96 weeks as a pilot trial because only 109 patients were enrolled and randomized at the end of the enrollment period despite a planned sample size of 240 patients, primarily due to the above mentioned negative reports of ABC use in the D:A:D study and ACTG 5202 (3, 8).

Materials and Methods

This clinical trial was designed and reported according to the recommendations of the Consolidated Standard of Reporting Trials (CONSORT) statement (22). The protocol and supporting CONSORT checklist are available as supplementary files (see Supplementary files 1 and 2).

Ethics statement

The Research Ethics Committee of each participating center approved the study protocol. All patients enrolled in this study provided a written informed consent. This study was conducted according to the principles expressed in the Declaration of Helsinki.

Study design

The Epzicom-Truvada study is a phase 4, multicenter, randomized, open-label, parallel group pilot study conducted in Japan that compared the efficacy and safety of a fixed dose of ABC/3TC and TDF/FTC, both combined with ritonavir-boosted atazanavir (ATV/r) for the initial treatment of HIV-1 infection for 96 weeks. Enrollment of patients began in November 2007 and ended in March 2010, and the follow-up period ended in February 2012. With a one to one ratio, the patients were randomly assigned to receive either a fixed dose of ABC/3TC or TDF/FTC, both administered with ATV/r. The randomization was stratified according to each participating site and conducted at the data center with

independent clinical research coordinators using a computer-generated randomization list prepared by a statistician with no clinical involvement in the trial.

Study patients

This study population included treatment-naïve Japanese patients aged 20 or over with HIV-1 infection who met the eligibility criteria for the commencement of antiretroviral therapy according to the DHHS Guidelines in place in the U.S. at the time of the writing of the study protocol (a CD4 count <350/μL or a history of AIDS-defining illness regardless of the CD4 count) (23). Patients were screened and excluded if they had previously taken lamivudine, tested positive for hepatitis B surface antigens, had comorbidities such as hemophilia or diabetes mellitus that required medical treatment, congestive heart failure or cardiac myopathy or if they were considered not suitable for enrollment by the attending physicians. Candidates were also excluded if their alanine aminotransferase level was 2.5 times greater than the upper limit of normal, they had an estimated glomerular filtration rate (eGFR) calculated using the Cockcroft-Gault equation of <60 mL/min, {creatinine clearance = [(140- age) × weight (kg)]/(serum creatinine ×72)(×0.85 for females)} or a serum phosphate level <2 mg/dL or had active opportunistic diseases that required treatment (24). Each patient's actual body weight was used for the calculation of eGFR. At screening, a genotypic drug resistant test and screening for the HLA-B*5701 allele were permitted but not required because the prevalence of both the drug resistant virus and the HLA-B*5701 allele are low in Japanese patients (7, 25). Medical history, including a history of AIDS-defining illnesses and other comorbidities, was also collected. Enrollment stopped on March 3, 2008 due to the recommendation from the DSMB of the trial based on the interim analysis of the ACTG5202 that ABC/3TC is less effective than TDF/FTC in patients with a baseline viral load >100,000 copies/mL (3). Accordingly, the DSMB recommended that the trial should be restarted with modified inclusion criteria: to enroll patients with an HIV-1 viral load of <100,000 copies/mL at screening, and the enrollment restarted from April 1, 2008.

Study procedures

Required visits for participants for clinical and laboratory assessments were at screening, enrollment and every 4 weeks until the viral load diminished to <50 copies/mL. For patients with a viral load <50 copies/mL, the required visit interval was every 12 weeks for the duration of the study. The evaluation performed at each visit included a physical examination, CD4 cell count, HIV-1 RNA viral load, a complete blood cell count and blood chemistries (total bilirubin, alanine aminotransferase, lactate dehydrogenase, serum creatinine, potassium, phosphate, triglycerides and low-density lipoprotein (LDL) cholesterol) and a urine examination of the levels of phosphate, creatinine and β2 microglobulin. The values of urinary β2 microglobulin were expressed relative to a urinary creatinine level of 1 g/L (/g Cr). The percent tubular resorption of phosphate was calculated using the following formula: {1-[(urine phosphate × serum creatinine)/(urine creatinine × serum phosphate)]} ×100 (26). All data, including the HIV-1 RNA viral load, were collected at each participating site and sent to the data center. Grade 3 or 4 serious adverse events were reported to the DSMB, which made a judgment whether they were caused by the study drugs. Independent research coordinators at the data center visited at least 10 facilities every year to monitor the accuracy of the submitted data and compliance to the study protocol. All authors vouch for the completeness and accuracy of the reported data.

Statistical analysis

The sample size calculation was originally conducted as follows: Assuming a 90% success rate in the TDF/FTC arm at week 48, a sample size of 224 patients (112 patients per arm) provided 80% power (one sided, α=0.05) to establish non-inferiority of ABC/3TC to TDF/FTC each in combination with ATV/r. Non-inferiority was defined as the lower bound of the two-sided 95% confidence interval (CI) with the treatment difference being above -10%. Based on this assumption, the targeted sample size was set to 240 patients (120 in each arm). However, as previously described, due to the shortage of accrued subjects, this study was underpowered and conducted as a pilot trial.

The primary efficacy endpoint was the time from randomization to virologic failure (defined as a confirmed HIV-1 RNA >1,000 copies/mL at or after 16 weeks and before 24 weeks or >200 copies/mL at or after 24 weeks) (3). The secondary efficacy endpoints included the time from randomization to either virologic failure or ART modification and a comparison of the proportions of patients with HIV-1 RNA <50 copies/mL at weeks 48 and 96 regardless of previous virologic failure. The intent-to-treat (ITT) population comprising all randomized subjects was used to assess the efficacy data; however, a comparison of the proportion of virologically-suppressed patients was conducted with both the ITT and a per protocol population while on the initial randomized regimen.

The safety endpoint was the time from randomization to the first occurrence of grade 3 or 4 laboratory data or abnormal symptoms that were at least one grade higher than the baseline. Isolated hyperbilirubinemia was excluded from the safety endpoints. The grade of adverse events was classified according to the Division of AIDS Table for grading the severity of adult and pediatric events, version 2004 (27). The tolerability endpoint was the time from randomization to any regimen modification. The safety and tolerability endpoints were calculated in the ITT population. Changes per protocol in the CD4 cell count, lipid markers and renal tubular markers at weeks 48 and 96 were compared using the Mann-Whitney test. A repeated measures mixed model was used to estimate and compare changes in the renal function between the two arms (17). The renal function was calculated using the Modification of Diet in Renal Disease study

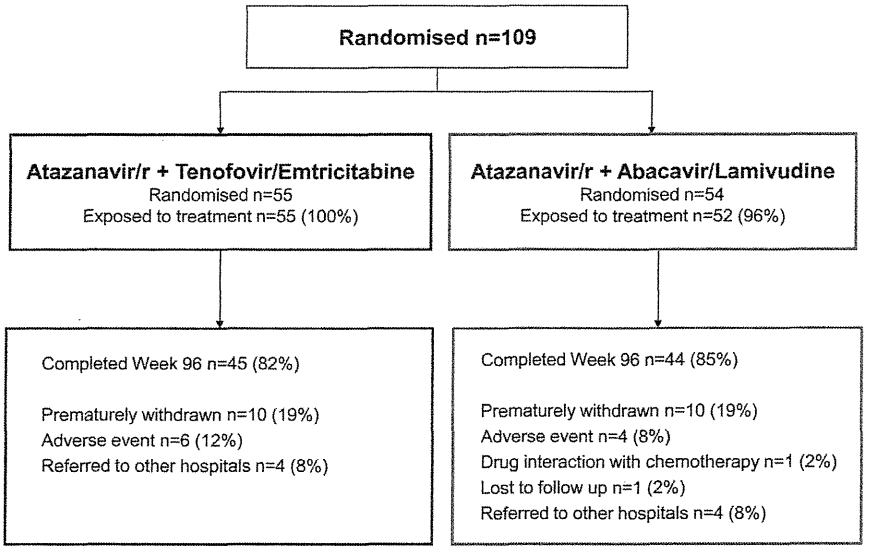

Figure 1. Enrollment, randomization and disposition of patients.

Table 1. Demographic and Baseline Characteristics

	ABC/3TC (n=54)	TDF/FTC (n=55)	Total (n=109)
Sex (male), n (%)	53 (98.1)	54 (98.2)	107 (98.2)
Age (years)†	39 (28.8-44)	35 (29-42)	36 (29-42.5)
CD4 count (/μL)†	236.5 (194-301.3)	269 (177-306)	257 (194-305)
HIV RNA viral load (log_{10}/mL)†	4.29 (3.92-4.67)	4.28 (3.86-4.60)	4.28 (3.89-4.67)
HIV RNA viral load >100,000 log_{10}/mL, n (%)	1 (1.9)	0 (0)	1 (0.9%)
Route of transmission (homosexual contact), n (%)	47 (87)	49 (89.1)	96 (88.1)
History of AIDS n (%)	1 (1.9)	5 (9.1)	6 (5.5)
Body weight (kg)†	64 (59-72.1)	63.1 (58-69)	64 (58.3-70.7)
Body mass index (kg/m^2)†	22.6 (20.4-24.2)	21.9 (20.3-23.6)	22.4 (20.3-23.7)
eGFR (mL/min/1.73 m^2)†	96.9 (82.7-107.3)	94.4 (83.6-105.7)	96.7 (83.0-106.7)
Creatinine clearance (mL/min)†	119.3 (105.4-136.6)	124.6 (103-139.3)	120.3 (104.7-138.3)
Serum creatinine (mg/dL)†	0.76 (0.67-0.83)	0.75 (0.68-0.84)	0.76 (0.68-0.83)
Urinary β2 microglobulin (μg/g Cre)†	195.8 (98.3-505.3)	138.4 (86.8-426.4)	172.9 (88.3-458.7)
Tubular resorption of phosphate (%)†	92.9 (90-95.1)	92.3 (87.7-95.2)	92.7 (89.3-95.1)
LDL-cholesterol (mg/dL)†	91.5 (75-125.5)	94 (72.5-111.5)	94 (74.5-114)
Triglycerides (mg/dL)†	132 (98-170.5)	114 (73-184)	127 (85.5-175)
Hypertension, n (%)	3 (5.6)	1 (1.8)	4 (3.7)
Diabetes mellitus, n (%)	0 (0)	0 (0)	0 (0)
Concurrent use of nephrotoxic drugs, n (%)	10 (18.5)	10 (18.2)	20 (18.3)
Hepatitis C, n (%)	0 (0)	0 (0)	0 (0)

†median (interquartile range)

IQR: interquartile range, AIDS: acquired immunodeficiency syndrome, eGFR: estimated glomerular filtration rate, LDL: low-density lipoprotein

equation adjusted for the Japanese population (28), and a sensitivity analysis was conducted using the above mentioned Cockcroft-Gault equation.

Time-to-event distributions were estimated using the Kaplan-Meier method and compared using the two-sided log-rank test. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were estimated using the Cox proportional hazards model. For grade 3 or 4 serious adverse events caused by the study drugs, the description and severities were recorded. Statistical significance was defined at two-sided p values <0.05. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Patient disposition and baseline characteristics

109 patients from 18 centers were enrolled and randomized between November 2007 and March 2010. Of these patients, 54 and 55 were allocated to the ABC/3TC and TDF/FTC arms, respectively (Fig. 1). The baseline demographics and characteristics are shown in Table 1. Most patients were men, with a median body weight of 64 kg. The median CD4 cell count was 257/μL (IQR: 194-305). One patient in the ABC/3TC arm had a baseline HIV-1 RNA level of >100,000

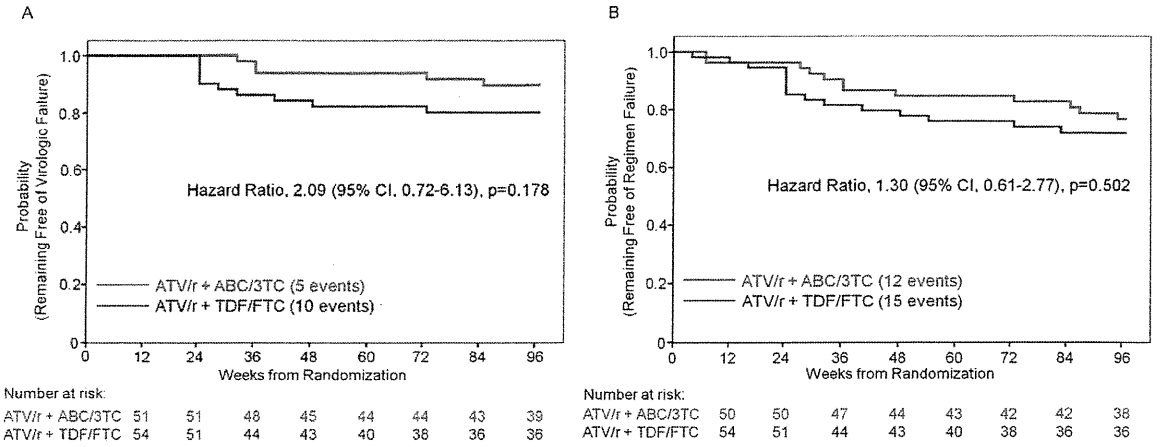

Figure 2. Efficacy results over 96 weeks. (A) Time to protocol-defined virologic failure. (B) Time to the first occurrence of either virologic failure or discontinuation of the initially randomized regimen. ATV/r: ritonavir-boosted atazanavir, ABC/3TC: abacavir/lamivudine, TDF/FTC: tenofovir/emtricitabine

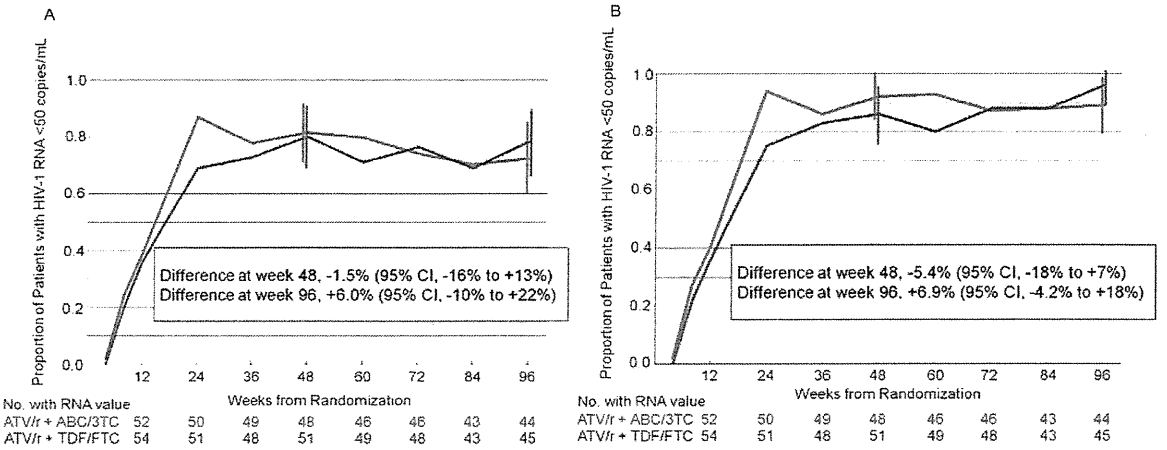

Figure 3. Efficacy results at 48 and 96 weeks. Proportion of patients with an HIV RNA level <50 copies/mL regardless of previous virologic failure with 95% binomial confidence intervals at 48 and 96 weeks. (A) Intention-to-treat analysis. (B) Per protocol analysis. ATV/r: ritonavir-boosted atazanavir, ABC/3TC: abacavir/lamivudine, TDF/FTC: tenofovir/emtricitabine

copies/mL. This patient was enrolled before the announcement of the interim analysis of ACTG5202 in March 2008 and achieved an HIV-1 RNA level of <50 copies/mL by the end of that month. One patient in the TDF/FTC arm had a history of lamivudine use. That patient was included in the analysis because this aspect of the medical history was identified after randomization and initiation of the allocated treatment.

Efficacy results

In the primary efficacy analysis, the time to virologic failure was not significantly different in the ABC/3TC arm from that observed in the TDF/FTC arm by 96 weeks (HR, 2.09; 95% CI, 0.72-6.13; p=0.178). Virologic failure occurred in 5 and 10 patients in the ABC/3TC and TDF/FTC arms, respectively (Fig. 2A). In the secondary efficacy analysis, the times to the first occurrence of confirmed virologic failure or discontinuation of the initially allocated regimen were not different between the two arms (HR, 1.30; 95% CI, 0.61-2.77; p=0.502) (Fig. 2B). Among the ITT population, the proportion of patients with an HIV RNA level <50 copies/mL at week 48 regardless of previous virologic failure was 81.5% in the ABC/3TC group and 80% in the TDF/FTC group, for a difference of -1.5% (95% CI, -16% to 13%), and at week 96, 72.2% and 78.2% for the ABC/3TC and TDF/FTC groups, respectively, for a difference of 6% (95% CI, -10% to 22%) (Fig. 3A). The per protocol analysis showed that the proportions at week 48 were 91.7% and 86.3% for the ABC/3TC and TDF/FTC groups, respectively, for a difference of -5.4% (95% CI, -18% to 7%). At week 96, the proportions were 88.6% and 95.6% for the ABC/3TC and TDF/FTC groups, respectively, for a

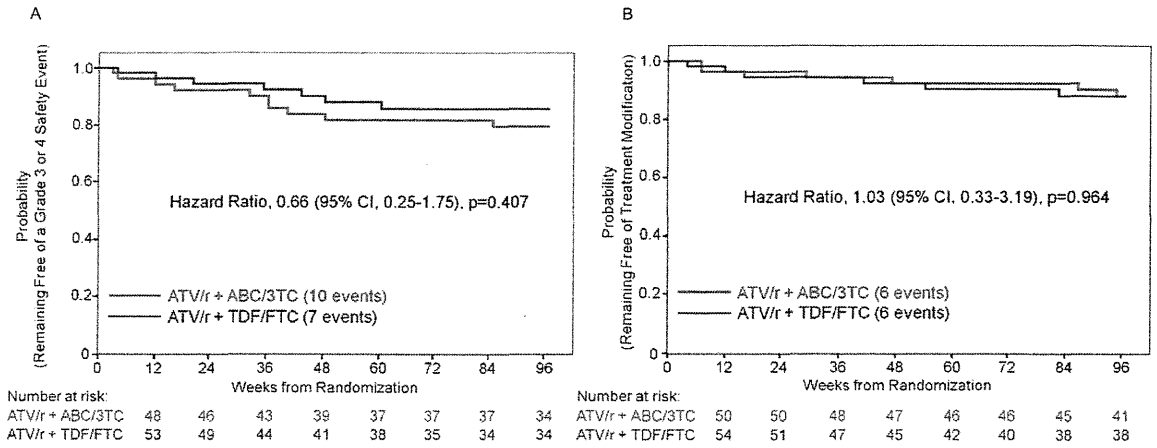

Figure 4. Safety and tolerability results over 96 weeks. (A) Time to first primary safety endpoint, defined as the first grade 3 or 4 event on the initial randomized regimen, which was at least one grade higher than baseline. (B) Time to tolerability endpoint, defined as the first change in regimen. ATV/r: ritonavir-boosted atazanavir, ABC/3TC: abacavir/lamivudine, TDF/FTC: tenofovir/emtricitabine

Table 2. Selected Grade 3 or 4 Events While Receiving Randomized Antiretroviral Drugs

	ABC/3TC (n=54)	TDF/FTC (n=55)	Total (n=109)
Overall, n (%)	13 (24)	10 (18)	23 (21)
Laboratory, n (%)	12 (22)	7 (13)	19 (17)
Alanine aminotransferase, n	0	1	1
LDL-cholesterol, n	6	2	8
Triglycerides, n	0	3	3
Uric acid, n	1	0	1
Serum phosphate, n	2	0	2
Serum calcium, n	1	0	1
Serum creatinine, n	1	0	1
Platelets count, n	1	1	2
Symptoms, n (%)	1 (2)	3 (5)	4 (4)
Depression, n	0	2	2
Fever, n	1	1	2

More than one event occurred in 2 patients.
LDL: low-density lipoprotein

difference of 6.9% (95% CI, -4.2% to 18%) (Fig. 3B). The primary and secondary efficacy analyses did not show a significant difference in viral efficacy between the two arms.

Safety and tolerability results

10 (18.5%) and 7 (12.7%) patients in the ABC/3TC and TDF/FTC groups, respectively, experienced 23 grade 3 or 4 adverse events related to the study drugs while on the initial regimen. The time to the first adverse event was not significantly different between the two arms (HR 0.66; 95% CI, 0.25-1.75, p=0.407) (Fig. 4A). Table 2 shows a list of selected grade 3 or 4 safety events. Among the adverse events, 48% included elevation of lipid markers. The tolerability endpoint, the time to first ART modification, was not significantly different between the two arms (HR 1.03; 95% CI, 0.33-3.19, p=0.964), and only 6 (11.1%) and 6 (10.9%) patients in the ABC/3TC and TDF/FTC arms, respectively, discontinued the initially allocated regimen by 96 weeks (Fig. 4B). The most common reason for regimen modification was drug toxicity (n=10; 4 in ABC/3TC and 6 in TDF/FTC arm; suspected ABC hypersensitivity reactions based on the appearance of rash and fever in HLA-B*5701-negative patient; n=1, depression; n=3, jaundice; n=3, nausea; n=2, and lipodystrophy; n=1). One patient in the ABC/3TC group developed a cerebral infarction during week 39 but was able to continue the study drugs. No deaths were registered during the study period.

Changes in the CD4 cell count and other parameters of interest

The increase in the median CD4 count from baseline to 48 weeks was marginally larger in the ABC/3TC arm than in the TDF/FTC arm (median: ABC/3TC: 216, TDF/FTC: 192, p=0.107). This difference was significantly larger at 96

Table 3. Median Values of Changes in Parameters of Interest from Baseline to 96 Weeks

	ABC/3TC (n=54)				TDF/FTC (n=55)				
	Number tested (baseline, week 96)	Baseline	Week 96	Median Δ	Number tested (baseline, week 96)	Baseline	Week 96	Median Δ	p value
CD4 cell count (/μL)	54, 43	236.5	545	328	55, 45	269	493	216	0.031
Lipids									
LDL-cholesterol (mg/dL)	54, 16	91.5	149	31.5	53, 16	94	97	2	0.026
Triglyceride (mg/dL)	54, 29	132	257	111	55, 26	114	202	40.5	0.037
Renal tubular markers									
Urinary β2 microglobulin (μg/g Cre)	49, 32	195.8	99.2	-94.9	52, 38	138.4	303.9	86.6	<0.001
Tubular resorption of phosphate (%)	49, 32	93	92	-1.4	50, 36	92	91	-2.6	0.930

LDL: low-density lipoprotein

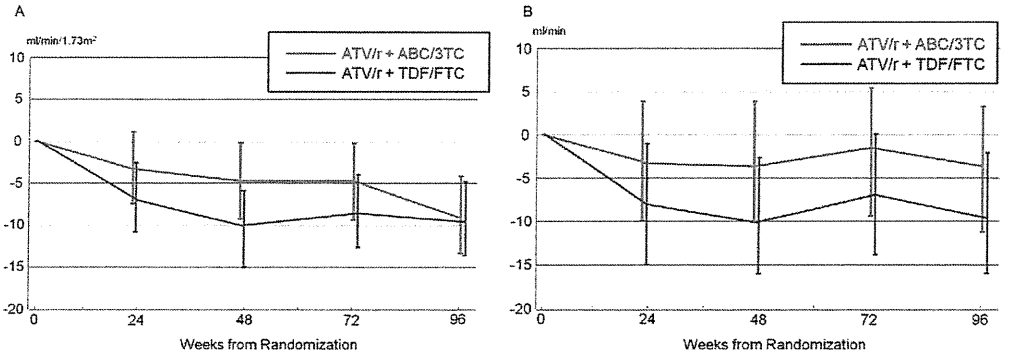

Figure 5. Changes in the renal function between baseline and 96 weeks. (A) Changes in the estimated glomerular filtration rate calculated with the Modification of Diet in Renal Disease study equation adjusted for the Japanese population. (B) Changes in creatinine clearance calculated with the Cockcroft-Gault equation. The data are presented as the mean±95% confidence interval. ATV/r: ritonavir-boosted atazanavir, ABC/3TC: abacavir/lamivudine, TDF/FTC: tenofovir/emtricitabine

weeks (ABC/3TC: 328, TDF/FTC: 236, p=0.031, Table 3). The increases in both LDL-cholesterol and triglycerides from baseline to 96 weeks were more significant in the ABC/3TC arm than in the TDF/FTC arm. One patient in the TDF/FTC arm had been treated with lipid-lowering medications prior to study enrollment. Furthermore, 7 patients and 1 patient in the ABC/3TC and TDF/FTC arms, respectively, started lipid-lowering agents during the study period. With regard to renal tubular markers, the levels of urinary β2 microglobulin increased in the TDF/FTC arm (median: 86.6 μg/g Cre), whereas it decreased in the ABC/3TC arm (median: -94.9 μg/g Cre). These changes were significantly different between the two arms (p<0.001). On the other hand, tubular resorption of phosphate did not show changes from baseline to 96 weeks in the two groups, and the levels were not different between the two arms (Table 3).

Changes in the renal function

A data analysis using repeated measures mixed models showed a significant decrease in the mean eGFR from baseline to 96 weeks in both groups (ABC/3TC: -8.7 mL/min/1.73 m^2, 95%CI -13.3 to -4.2, p<0.001; TDF/FTC: -9.2 mL/min/1.73 m^2, 95%CI -13.7 to -4.7, p<0.001) (Fig. 5A). There was no significant interaction between the trend of the two arms over time (p=0.202), thus indicating that the change in eGFR from baseline to 96 weeks was not significantly different between the two arms. A sensitivity analysis of creatinine clearance calculated using the Cockcroft-Gault equation showed that creatinine clearance decreased significantly from the baseline in the TDF/FTC arm (-9.6 mL/min, 95%CI -16.6 to -2.5, p<0.001) but not in the ABC/3TC arm (-4.1 mL/min, 95%CI -11.2 to 3.0, p=0.466)(Fig. 5B). No significant interaction between the trend of the two arms was observed with respect to creatinine clearance (p=0.403). Two patients in the ABC/3TC arm progressed to more advanced chronic kidney disease (CKD) stage by the last per protocol visit: one patient progressed to stage 4 CKD (eGFR <30 mL/min/1.73 m^2) and the other to stage 3 CKD (eGFR <60 mL/min/1.73 m^2). However, ABC/3TC did not appear to be the causative drug for renal dysfunction in these two cases because the deterioration in the renal function was associated with the development of malignant lymphoma in the former patient and with the commencement of fenofibrate treatment in the latter; renal function recovered rapidly in the latter patient after the discontinuation of fenofibrate.

Discussion

Although insufficiently powered to show the non-inferiority of the viral efficacy of ABC/3TC relative to TDF/

FTC, this pilot study is the first randomized study conducted in Asia to elucidate the efficacy and safety of fixed doses of these two regimens each administered in combination with ATV/r for initial HIV-1 therapy. Viral efficacy, safety, and tolerability were not significantly different in the two arms of Japanese patients with a baseline HIV viral load <100,000 copies/mL over 96 weeks. Both regimens showed favorable viral efficacy, as in the ITT population, 72.2% and 78.2% of the patients in the ABC/3TC and TDF/FTC arms, respectively, had HIV-1 viral loads of <50 copies/mL at 96 weeks. Both regimens were also well-tolerated, as only 11.1% and 10.9% of the patients in the ABC/3TC and TDF/FTC arms, respectively, discontinued the allocated regimen by 96 weeks. Clinically suspected (not immunologically-confirmed) ABC-associated hypersensitivity reaction occurred in only one (1.9%) patient in the ABC/3TC arm, confirming that ABC hypersensitivity is rare in populations in which HLA-B*5701-positive patients are uncommon. Thus, this trial suggests that ABC/3TC may be an efficacious and safe regimen for use in HLA-B*5701-negative populations, such as the Japanese, with a baseline HIV viral load <100,000 copies/mL.

The usefulness of ABC/3TC has recently received higher recognition for two reasons. One, a meta-analysis by the FDA did not confirm the association between ABC use and myocardial infarction (9). Two, it became clear that TDF-induced renal tubulopathy results in decreased bone mineral density due to phosphate wasting and a decreased renal function, both of which might develop into serious complications with long-term TDF use (12-14, 29, 30). On the other hand, greater deteriorations in the levels of lipid markers were noted in ABC/3TC than in TDF/FTC in clinical trials comparing these two agents (16, 17). The present study also demonstrated that the increases in the LDL-cholesterol and triglyceride levels were higher in the ABC/3TC arm than in the TDF/FTC arm.

TDF-induced nephrotoxicity is of particular interest in this study because a low body weight is an important risk factor, and body stature was much smaller in this study population (median baseline body weight 64 kg), than in the ASSERT study (72 kg), which compared the renal function between patients receiving ABC/3TC and TDF/FTC with efavirenz in Europe (17, 18, 20). This study showed that changes in the renal function from baseline were not significantly different between the two arms, similar to the findings of the ASSERT study. None of the patients in the TDF/FTC arm exhibited progression of CKD stage. On the other hand, the levels of urinary β2 microglobulin deteriorated significantly from baseline in the TDF/FTC arm, whereas improvements were observed in the ABC/3TC arm. This is also similar to the findings reported by the ASSERT trial. This suggests that urinary β2 microglobulin is a more sensitive marker for evaluating TDF nephrotoxicity than the renal function calculated by serum creatinine, as also demonstrated in our previous work (31). Tubular resorption of phosphate, another marker examined to evaluate the renal tubular function, did not exhibit any changes from baseline or between the two arms, suggesting that urinary β2 microglobulin may be a better marker for evaluating TDF nephrotoxicity than tubular resorption of phosphate. Of note, in both arms, the renal function did significantly decrease from baseline. To our knowledge, this is the first randomized trial comparing ABC/3TC and TDF/FTC that observed deterioration of the renal function after the initiation of ART. This result highlights the importance of regular monitoring of renal function after initiation of ART, although it is difficult to draw a firm conclusion on the prognosis of the renal function from this study, due to the limited length of the observation period and the small number of enrolled patients.

Only one patient (1.9%) in the ABC/3TC arm developed a clinically suspected ABC-associated hypersensitivity reaction, which was diagnosed based on the appearance of a skin rash and fever six weeks after commencement of the study drug. The patient fully recovered after discontinuation of the drugs. The ASSERT trial of HLA-B*5701-negative patients reported a similar incidence (3%) of clinically suspected ABC hypersensitivity reactions (17). The one case observed in our trial could be a false positive, because ABC hypersensitivity reactions commonly occur 9-11 days after the initiation of therapy (32), and ABC hypersensitivity was not confirmed immunologically. Nonetheless, immediate discontinuation of ABC is highly recommended even in HLA-B*5701-negative patients suspected of ABC hypersensitivity, since ABC hypersensitivity can occur in such patients (33) and errors in genotyping for HLA or reporting a genotype might occur in practice (34).

Several limitations of this trial should be acknowledged. First, due to the shortage of enrolled patients, the trial was insufficiently powered to test non-inferiority of the viral efficacy of ABC/3TC against TDF/FTC, as initially planned. However, the safety and tolerability data of these regimens in Asia are a valuable asset for patients from this region, and efficacy data could be utilized as part of a meta-analysis in the future. Second, the enrolled subjects were mostly men (primarily men who had sex with men and very few injection drug users). Further studies are needed to examine the efficacy and safety of these regimens in women and patients with different routes of transmissions in Asia.

In summary, this randomized trial demonstrated high efficacy and safety of fixed-dose ABC/3TC and TDF/FTC in combination with ATV/r over 96 weeks for treatment-naïve Japanese patients with a baseline HIV-1 viral load <100,000 copies/mL, although it was insufficiently powered to show non-inferiority of the viral efficacy of ABC/3TC compared with TDF/FTC. ABC/3TC with ATV/r is a safe and efficacious initial regimen for treating HLA-B*5701-negative patients with a baseline HIV-1 viral load <100,000 copies/mL.

Author's disclosure of potential Conflicts of Interest (COI).
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Authors' contributions

SO, MT (Takano), MI, HG, YK and YT designed the study. TE, MH, SK, HU, TK, TN (Naito), MY (Yoshida), NT, MU, YY, TF, SH, KT, MY (Yamamoto), SM, MT (Tateyama) and YT collected the data. HM supervised the study and reviewed and approved study report. TN (Nishijima), HK, HG and SO analyzed and interpreted the data. TN (Nishijima), HK, HG and SO drafted the manuscript and all other authors revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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Prophylactic Effect of Antiretroviral Therapy on Hepatitis B Virus Infection

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Background. Hepatitis B virus (HBV) infection is common in individuals infected with human immunodeficiency virus, especially in men who have sex with men (MSM). Almost all currently used regimens of antiretroviral therapy (ART) contain lamivudine (LAM) or tenofovir disoproxil fumarate (TDF), both of which have significant anti-HBV activity. However, the prophylactic effect of ART on HBV infection has not been assessed previously.

Methods. Non-HBV-vaccinated HIV-infected MSM were serologically evaluated for HBV infection using stocked serum samples. Cases negative for HBV surface antigen (HBsAg), antibody to HBsAg (anti-HBs), and antibody to HBV core antigen (anti-HBc) in first serum samples were serologically followed until last available stocked samples. HBV genotype and LAM-resistant mutation (rtM204V/I) were analyzed in cases that became HBsAg-positive.

Results. The first stocked samples were negative for all analyzed HBV serological markers in 354 of 1434 evaluated patients. The analysis of their last samples indicated HBV incident infection in 43 of them during the follow-up period. The rate of incident infections was lower during LAM- or TDF-containing ART (0.669 incident infections in 100 person-years) than during no ART period (6.726 incident infections in 100 person-years) and other ART (5.263 incident infections in 100 person-years) ($P < .001$). Genotype A was most prevalent (76.5%), and LAM-resistant HBV was more frequent in incident infections during LAM-containing ART (50.0%) than in those during no ART and other ART (7.1%) ($P = .029$).

Conclusions. LAM- and TDF-containing ART regimens seem to provide prophylaxis against HBV infection, although drug-resistant strains seem to evade these effects.

Keywords. lamivudine; tenofovir disoproxil fumarate; resistant; chronic infection.

Patients with human immunodeficiency virus (HIV) infection are at high risk for both hepatitis B virus (HBV) infection and development of chronic infection [1–4]. Based on information from Western countries, the rate of coinfection varies according to risk categories; the highest rate is in men who have sex with men (MSM), with a slightly lower rate among intravenous drug users, and much lower in individuals infected through heterosexual contacts [5–8]. In Japan, HIV/HBV coinfection is also significantly associated with MSM [9, 10]. The progression of chronic HBV infection to cirrhosis, end-stage liver diseases, and/or hepatocellular carcinoma is more rapid in HIV-infected persons than in those with chronic HBV infection alone [11, 12]. Vaccination of non-HBV-immunized HIV-infected individuals is recommended to prevent HBV infection [13]. However, all current recommended antiretroviral therapy (ART) regimens contain lamivudine (LAM) or tenofovir disoproxil fumarate (TDF), both of which have significant anti-HBV activity [14]. Do these ART regimens provide any prophylaxis against HBV infection? This is an important question, as a positive answer could influence the strategy applied to prevent HBV infection in HIV-infected individuals. To delineate the hepatitis B prophylactic effect of ART, we used stocked samples for serological evaluation of HBV infection in HIV-infected MSM. The present

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study included those patients who had tested negative for hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc) using their first stocked blood samples, who were followed up serologically to identify new HBV incident infections among them. The other part of the study covered analysis of the relation between the frequency of incident infection and ART regimens.

METHODS

Patients

Since April 1997, we have stocked serum samples taken at routine clinical practice from HIV type 1 (HIV-1)–infected patients who visited the Outpatient Clinic of the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, under signed informed consent for use in virologic research. Every patient had been interviewed at the first visit by clinical nurse specialists at the HIV outpatient clinic using a structured questionnaire that includes items on sexuality and history of HBV vaccination. Most of the patients regularly visited our clinic every 1–3 months, and we had collected and stored their sera at almost all visits. The ethics committee of the National Center for Global Health and Medicine approved the collection and analysis of the samples. First, we selected HIV-1–infected MSM who met the following inclusion criteria: (1) the first visit to our clinic was between April 1997 and December 2009, (2) they had not received HBV vaccination before the first visit, and (3) at least 2 serum samples were available and collected at least 6 months apart. The first sample was defined as the baseline serum sample, and baseline clinical data were defined as those recorded on the date of sampling of the first stocked serum. Patients' baseline characteristics, including age, race, hepatitis C virus antibody, results of *Treponema pallidum* hemagglutination assay, and $CD4^+$ cell count were collected from the medical records.

HBV Analysis

In order to identify new HBV incident infection, we excluded patients with previously confirmed HBV infection. The baseline samples of the patients who met the inclusion criteria described above were serologically evaluated for HBsAg, anti-HBs, and anti-HBc using ARCHITECT HBsAg QT assay, anti-HBs assay, and anti-HBc assay, respectively (Abbott Laboratories, Chicago, Illinois) [15, 16]. Patients positive for any of HBsAg, anti-HBs, and anti-HBc at baseline were excluded from the serological follow-up. The remaining patients were considered to have never been infected with HBV before the baseline. Their last stocked sample taken before or in December 2010, or before HBV vaccination if performed during the follow-up period, was analyzed for HBsAg, anti-HBs, and anti-HBc. If the last sample was negative for all 3, the patient was considered to have never been infected with HBV up to the sampling date of the last stocked serum. If HBsAg, anti-HBs, or anti-HBc was positive in the last stocked serum, the patient was considered to have HBV incident infection during the follow-up period. In the latter case, the baseline samples were subjected to polymerase chain reaction (PCR) analysis for HBV DNA [17, 18], and all the stocked samples during the follow-up period were serologically analyzed to determine the date of HBV incident infection. The date of incident infection was defined as the sampling date of the first positive serum for any HBV serological marker. The time from the baseline to HBV incident infection was analyzed by the Kaplan-Meier method. The data were censored at the sampling date of the last stocked sample if it was negative for all analyzed HBV serological markers. Patients' age and $CD4^+$ cell count at the date of incident infection and alanine aminotransferase (ALT) values within 3 months of incident infection were collected. If an HBsAg-positive sample was available, HBV genotype and LAM-resistant mutation (rtM204V/I) were analyzed by PCR-invader assay [17–19]. The diagnosis of chronic HBV infection was considered when HBsAg was still positive in sera taken at 6 months or longer after the incident infection.

Antiretroviral Therapy

To determine the type of ART under which HBV incident infection occurred, the regimen information of ART was collected from medical records over the period spanning from the baseline to the incidence infection or to the end of follow-up. The treatment status was divided into 4 categories: (1) No ART, no treatment with any antiretroviral agent; (2) Other-ART, ART with regimens that did not contain LAM, TDF, or emtricitabine (FTC); (3) LAM-ART, ART with LAM-containing regimens that did not contain TDF or FTC; and (4) TDF-ART, ART with TDF-containing regimens with or without LAM or FTC. Data were censored on the sampling date of the last stocked sample if it was negative for all analyzed HBV serological markers. When the treatment category was modified, the data were censored on the date of category change for the previous treatment category and a new follow-up as a different case was initiated for the replacement treatment category.

Statistical Analysis

The time from the baseline to HBV incident infection was analyzed by the Kaplan-Meier method. The Cox proportional hazards regression analysis was used to assess the risk of HBV incident infections. The impact of patients' baseline characteristics, year of entry, the use of antiretroviral agents (any antiretroviral, and any of LAM, TDF, or FTC), and the frequency of changing ART regimen during the follow-up period was estimated with univariate analysis, and those with statistical significance were incorporated into multivariate analysis. The

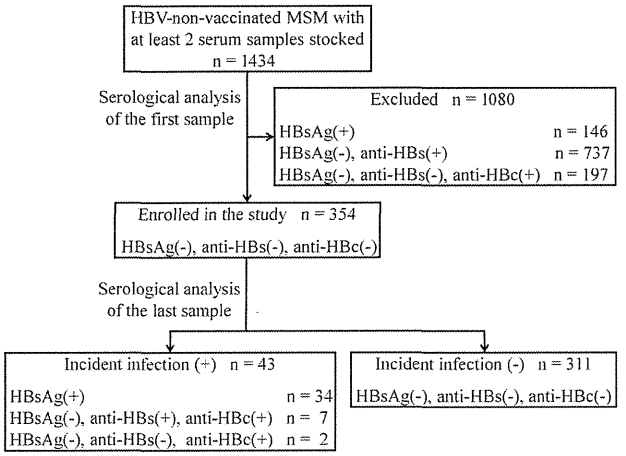

Figure 1. Patient selection process: 1434 patients met the inclusion criteria. Of these patients, 1080 were excluded because of positive hepatitis B virus serology in the first samples. The results of various serological tests are shown. The remaining 354 were enrolled for serological follow-up. Of these, 43 were positive in the last sample analysis. Their stocked samples were analyzed serologically and the results of HBV serology using the first positive samples are indicated. Abbreviations: anti-HBc, antibody to HBV core antigen; anti-HBs, antibody to HBsAg; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; MSM, men who have sex with men.

frequency and risk of HBV incident infection during each treatment category was also assessed by univariate Cox proportional hazards regression analysis. We used hazard ratios and 95% confidence intervals to estimate the impact of each variable on incident infection. Patients' age and $CD4^+$ cell count on the date of incident infection, and peak value of ALT within 3 months of incident infection were compared between transient infection and chronic infection with Wilcoxon rank-sum test. The differences in rates of HBV genotype A and rtM204V/I mutation were compared with χ^2 test (ie, the Fisher exact test). Statistical significance of difference was defined as a 2-sided *P* value of <.05. All statistical analyses were performed with the Statistical Package for Social Sciences version 17.0 (SPSS, Chicago, Illinois).

RESULTS

Figure 1 shows the patient selection procedure. A total of 1434 HIV-1–infected MSM met the inclusion criteria described in the Methods section. Of these, 146 patients (10.2%) were positive for HBsAg, 737 (51.4%) were positive for anti-HBs, and 197 (13.7%) were solely positive for anti-HBc using baseline samples. The remaining 354 patients (24.7%; negative for HBsAg, anti-HBs, and anti-HBc at baseline), who were considered to have never been infected with HBV, were enrolled for serological follow-up. Table 1 lists their baseline characteristics. Serological analysis of the last sample of each of these patients showed HBV incident infection during follow-up in 43 (12.1%). Their baseline samples were found to be PCR-negative for HBV DNA, confirming that the incident infection in these patients occurred during the follow-up period. All stocked samples of the 43 patients were analyzed serologically to determine the date of HBV incident infection. HBV incident infections occurred every year between 1997 and 2010 except in 1998. The median time period from the baseline to HBV incident infection was 1.6 years (interquartile range [IQR], 192–1151 days; range, 28–4068 days). The total observation period was 1607 person-years (median, 3.7 years [IQR], 1.9–6.5 years). Figure 2 shows the Kaplan-Meier curve for the HBV incident infection for the whole cohort of enrolled patients.

In order to assess the risk of HBV incident infections, patients' baseline characteristics, year of entry, the use of any antiretroviral agents, the use of any of LAM, TDF, or FTC, and the frequency of changing ART regimen during the follow-up

Table 1. Baseline Characteristics of the 354 Enrolled Patients

Characteristic	Total (n = 354)	Year of Entry			
		1997–2000 (n = 61)	2001–2003 (n = 79)	2004–2006 (n = 112)	2007–2009 (n = 102)
Age, y, median (IQR)	32.0 (27.0–38.0)	32.0 (27.8–37.3)	31.0 (27.0–37.8)	32.0 (27.0–38.0)	35.0 (27.0–42.0)
Race/ethnicity					
Japanese	340 (96.0)	59 (96.7)	78 (98.7)	109 (97.3)	94 (92.2)
Asian other than Japanese	4 (1.1)	0 (0.0)	0 (0.0)	1 (0.9)	3 (2.9)
Caucasian	10 (2.8)	2 (3.3)	1 (1.3)	2 (1.8)	5 (4.9)
HCV antibody, positive	8 (2.3)	1 (1.6)	2 (2.5)	1 (0.9)	4 (3.9)
TPHA positive	101 (28.5)	23 (37.7)	20 (25.3)	30 (26.8)	28 (27.5)
$CD4^+$ cell count, cells/mm^3, median (IQR)	277 (151–404)	277 (169–417)	313 (97–443)	316 (176–413)	252 (129–359)
HIV RNA, log_{10} copies/mL, median (IQR)	4.6 (3.8–5.2)	4.5 (3.6–5.2)	4.8 (3.9–5.4)	4.4 (3.8–4.9)	4.7 (3.9–5.2)

Data are No. (%) unless otherwise specified.

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; TPHA, *Treponema pallidum* hemagglutination assay.

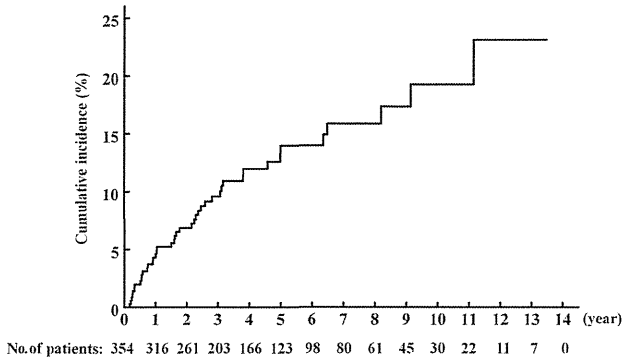

Figure 2. Kaplan-Meier curve showing the time to hepatitis B virus incident infection.

period were estimated using a proportional hazards model (Table 2). Younger age and higher $CD4^+$ cell count correlated positively, and use of any antiretroviral, use of LAM, TDF, or FTC, and the frequency of changing ART regimen correlated negatively with HBV incident infection, with statistical significance in univariate analysis. However, in multivariate analysis, the use of LAM, TDF, or FTC continued to show significant relation. Then, we focused on the relation between treatment status and HBV incident infection. The observation period in each patient was divided into 4 categories by treatment status: No ART, no treatment with any antiretroviral agent; Other-ART, ART with regimens that did not contain LAM, TDF, or FTC; LAM-ART, ART with LAM-containing regimens that did not contain TDF or FTC; or TDF-ART, ART with TDF-containing regimens with or without LAM or FTC. No participant received FTC single tablet (Emtriva). All the participants who took FTC received the combination tablet of TDF/FTC (Truvada), and therefore, such treatment status was categorized as TDF-ART. The total categorized observation period of No ART, Other-ART, LAM-ART, and TDF-ART was 446, 114, 814, and 233 person-years, respectively. The number of the HBV incident infections was 30 during the No ART period, 6 during Other-ART period, 7 during LAM-ART period, and 0 during TDF-ART period. No incident infection occurred at the time of changing ART regimen. The proportional hazards model showed a significantly lower frequency of HBV incident infection during LAM- or TDF-ART (0.669 incident infections per 100 person-years) compared with that during No ART (6.726 incident infections per 100 person-years), although there was no significant difference between Other-ART (5.263 incident infections per 100 person-years) and No ART, suggesting that ART regimens with anti-HBV activity can reduce HBV incident infections by 90% (Table 3). During LAM-ART, the HIV-1 load around the period of incident infection remained below the detection limit in all the 7 infected patients, indicating excellent adherence to ART.

Figure 3 shows peak ALT levels for the 43 HBV incident infections. Among the 36 incident infections observed the No ART and Other-ART groups, 16 infections (44.4%) were asymptomatic and not associated with significant increases in ALT (peak ALT, <60 IU/L). We were able to serologically follow 33 of the 36 cases for 6 months after the date of incident infection (TDF-ART was introduced within 6 months of incident infection in the other 3 cases). Among the 33 patients, 13 (39.4%) developed chronic infection (HBsAg was still positive 6 months after the date of incident infection). The median $CD4^+$

Table 2. Cox Proportional Hazards Regression Analysis for the Risk of Hepatitis B Virus Incident Infection

	Univariate Analysis		Multivariate Analysis	
Factors	Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value
Year of entry, per 1 y increase	.942 (.860–1.033)	.207		
Baseline characteristics				
Age, per 1 y increase	.921 (.879–.965)	.001	.958 (.917–1.001)	.054
Race (Japanese)	21.243 (.010–45 657.613)	.435		
HCV antibody	.048 (<.001–346.311)	.503		
TPHA	1.475 (.792–2.747)	.220		
$CD4^+$ cell count, per 100 cells/mm^3 increase	1.121 (1.008–1.246)	.035	.882 (.752–1.034)	.121
HIV RNA, per 1 $\log_{10}$ copies/mL increase	1.387 (.999–1.924)	.051		
Antiretroviral use during follow-up period				
Any antiretroviral	.097 (.052–.184)	<.001	.927 (.305–2.818)	.893
LAM, TDF, or FTC	.075 (.039–.146)	<.001	.110 (.031–.390)	.001
Frequency of changing regimen	.245 (.145–.414)	<.001	.700 (.385–1.270)	.240

Abbreviations: CI, confidence interval; FTC, emtricitabine; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LAM, lamivudine; TDF, tenofovir disoproxil fumarate; TPHA, *Treponema pallidum* hemagglutination assay.

Table 3. Frequency and Hazard Ratio of Hepatitis B Virus Incident Infection in Each Treatment Status Category

ART	Observation Period (Person-Years)	Incident Infection	Hazard Ratio (95% CI)	*P* Value
No ART	446	30	1	. . .
Other-ART	114	6	.924 (.381–2.239)	.861
ART containing at least 1 of LAM, TDF, and FTC[a]	1047	7	.113 (.049–.261)	<.001
LAM-ART	814	7		
TDF-ART	233	0		

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; FTC, emtricitabine; LAM, lamivudine; TDF, tenofovir disoproxil fumarate; LAM-ART, ART with LAM-containing regimens that did not contain TDF or FTC; Other-ART, ART with regimens that did not contain LAM, TDF, or FTC; TDF-ART, ART with TDF-containing regimens with or without LAM or FTC.

[a] No participant received FTC single tablet (Emtriva) during the observation period. All the participants who took FTC received the combination tablet of TDF/FTC (Truvada), and therefore, such treatment status was categorized into TDF-ART.

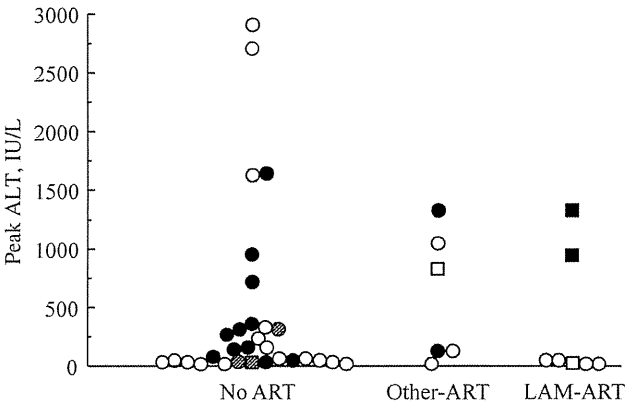

Figure 3. Peak alanine aminotransferase (ALT) values in hepatitis B virus (HBV) incident infections according to treatment regimen. Thirty, 6, and 7 HBV incident infections were observed during No antiretroviral therapy (ART), Other-ART, and lamivudine (LAM)–ART, respectively. No incident infection was identified during tenofovir disoproxil fumarate (TDF)–ART. No participant received emtricitabine (FTC) single tablet (Emtriva) during the observation period. All the participants who took FTC received the combination tablet of TDF/FTC (Truvada), and therefore, such treatment status was categorized into TDF-ART. Data are peak ALT values measured within 3 months of the date of incident infections. LAM-resistant mutation (rtM204V/I) was analyzed in 34 cases using the available hepatitis B surface antigen (HBsAg)–positive samples. Open squares: patients infected with LAM-resistant HBV. Closed circles and squares: patients who developed chronic infection (HBsAg-positive 6 months after the date of incident infection). Checked circles and squares: patients who received TDF-containing ART within 6 months of incident infection. Abbreviations: ALT, alanine aminotransferase; ART, antiretroviral therapy; LAM, lamivudine.

cell count was lower in the patients who developed chronic infection than in those with transient infection, although the difference was not significant ($P = .068$; Table 4), indicating that HIV-related immunodeficiency may play a role in the induction of chronic HBV infection. Among the 7 incident infections observed during LAM-ART, only 2 patients (28.6%) were symptomatic, had significant rise in ALT, and developed chronic HBV infection, and both of these infections were caused by LAM-resistant HBV (Table 5). The other 5 cases were asymptomatic and transient. Three of them were caused by LAM-sensitive strains and 1 was by LAM-resistant strain. HBsAg-positive serum sample was not available in the last case. LAM-resistant HBV was more frequently identified in analyzed incident infections during LAM-containing ART (50.0%) than in those during no ART and other ART (7.1%) ($P = .029$). Considered together, LAM seems to prevent acquisition of HBV infection, progression to symptomatic hepatitis, and development of chronic infection even after the development of infection, although these effects may be less pronounced in patients with LAM-resistant strains.

Among the 43 infection cases observed during total serological follow-up, HBsAg-positive samples were available in 34 cases and their HBV genotype was determined. Genotype A was the most frequent, as reported previously [10, 20–22], and genotypes B, G, and H were also identified. The rate of development of chronic infection was higher in genotype A than in other genotypes as previously reported [23], although the difference was not significant in our study. In the remaining 9 cases, only anti-HBc with (7 cases) or without (2 cases) anti-HBs were detected, although their samples were available and serologically analyzed at least every 3 months around the incident infection.

DISCUSSION

The results of this serological follow-up study indicated that LAM- and TDF-containing ART regimens protect against HBV incident infection. Furthermore, the results also suggested that LAM prevents progression to symptomatic hepatitis and development of chronic infection even after the development of HBV incident infection, provided such infection is caused by LAM-sensitive strains. However, it seems that LAM-resistant strains may evade this protective effect. One previous study that estimated the incidence of acute HBV infection among HIV-infected patients reported similar frequencies in patients receiving ART with and without LAM [5]. However, the authors defined immunoglobulin M anti-HBs positivity as a marker of HBV incident infection and did not exclude anti-HBc–positive patients at study entry. This probably made it difficult to distinguish incident infection from reactivation of chronic infection, as discussed in the report. In this study, we identified a

Table 4. Patient Characteristics and Clinical Features of Hepatitis B Virus Incident Infections in the No Antiretroviral Therapy (ART) and Other-ART Treatment Categories

Factors	Transient (n = 20)	Chronic[a] (n = 13)	Treated[b] (n = 3)	*P* Value[c]
Age, y, median (IQR)	31.0 (28.0–33.0)	29.0 (25.0–38.3)	25.0 (21.0–35.0)[d]	.406
$CD4^+$ cell count, cells/mm^3, median (IQR)	371 (308–518)	320 (235–383)	674 (206–1935)[d]	.068
Peak ALT level[e], U/L, median (IQR)	65 (30–573)	264 (115–774)	31 (15–314)[d]	.162
HBV genotype, No. (%)				.645
Genotype A	9 (45.0)	11 (84.6)	2 (66.7)	
Other genotypes	3 (15.0)	2 (15.4)	1 (33.3)	
Genotype unknown	8 (40.0)	0 (0.0)	0 (0.0)	
HBV rtM204V/I mutation, No. (%)				.480
Positive	1 (5.0)	0 (0.0)	1 (33.3)	
Negative	11 (55.0)	13 (100.0)	2 (66.7)	
Unknown	8 (40.0)	0 (0.0)	0 (0.0)	

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; IQR, interquartile range.

[a] Hepatitis B surface antigen–positive 6 months after the date of incident infection.

[b] Treated cases with tenofovir disoproxil fumarate–containing antiretroviral therapy within 6 months of incident infection.

[c] *P* values between transient and chronic cases calculated with Wilcoxon rank-sum tests for continuous variables and χ^2 tests for proportions.

[d] Minimum and maximum values.

[e] Peak ALT level within 3 months of incident infection.

significant number of isolated anti-HBc–positive patients, a finding in agreement with previous reports [24–27], and excluded them from the serological follow-up to avoid improper inclusion of isolated anti-HBc–positive ones as HBV-naive [28, 29].

Table 5. Patient Characteristics and Clinical Features of Hepatitis B Virus Incident Infections During LAM-ART Treatment

Factors	Transient (n = 5)	Chronic[a] (n = 2)	*P* Value[b]
Age, y, median (IQR)	33.0 (30.3–36.5)	38.0 (33.0–43.0)[c]	.329
$CD4^+$ cell count, cells/mm^3, median (IQR)	430 (267–648)	362 (360–364)[c]	.699
Peak ALT level[d], U/L, median (IQR)	22 (14–51)	1133 (941–1325)[c]	.051
HBV genotype, No. (%)			>.999
Genotype A	3 (60.0)	1 (50.0)	
Other genotypes	1 (20.0)	1 (50.0)	
Genotype unknown	1 (20.0)	0 (0.0)	
HBV rtM204V/I mutation, No. (%)			.400
Positive	1 (20.0)	2 (100.0)	
Negative	3 (60.0)	0 (0.0)	
Unknown	1 (20.0)	0 (0.0)	

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; IQR, interquartile range; LAM-ART, ART with LAM-containing regimens that did not contain TDF or FTC.

[a] Hepatitis B surface antigen–positive 6 months after the date of incident infection.

[b] *P* values calculated with Wilcoxon rank-sum tests for continuous variables and χ^2 tests for proportions.

[c] Minimum and maximum values.

[d] Peak ALT level within 3 months of incident infection.

HBV vaccination is recommended for individuals seeking evaluation or treatment for sexually transmitted diseases, HIV-infected patients, sexually active persons with >1 partner, and MSM [13]. However, the response and durability of adequate titers of anti-HBs are often reduced in HIV-infected patients [30–34]. Modified regimens of vaccination have been reported to improve anti-HBs response in HIV-infected patients, although the response rate was still low in those with low $CD4^+$ cell counts [35–37]. Our study demonstrated the HBV prophylactic effects of LAM- and TDF-containing ART regimens, suggesting that ART should be initiated before HBV vaccination, especially in those with low $CD4^+$ cell counts. Early introduction of ART was recommended recently not only for HIV-infected individuals, but also for prevention of transmission to others [38, 39]. Early introduction of treatment may also be recommended to prevent HBV infection to the patients themselves if they are HBV-naive. One randomized clinical trial reported the prophylactic effect of TDF combined with FTC in HIV prevention in seronegative MSM [40]. However, in that trial, HBV vaccination was offered to all susceptible participants, which made it impossible to estimate the prophylactic effect of the treatment on HBV prevention.

Our study carries certain limitations related to its retrospective nature. Patients on ART might have more opportunities to improve their behavior to prevent transmission of HIV to others, which could reduce HBV infection in themselves but

introduce bias in our analysis. However, the results suggest prophylaxis against potential HBV infection by oral medications, which could be useful for nonimmunized medical care providers.

Notes

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Potential conflicts of interest. H. G. has received honoraria from MSD K.K., Abbott Japan, Janssen Pharmaceutical K.K., Torii Pharmaceutical, and ViiV Healthcare. S. O. has received honoraria and research funding from MSD K.K., Abbott Japan, Janssen Pharmaceutical K.K., Pfizer, Roche Diagnostics K.K., and ViiV Healthcare, and has received honoraria from Astellas Pharmaceutical K.K., Bristol-Myers K.K., Daiichisankyo, Dainippon Sumitomo Pharma, GlaxoSmithKline, K.K., Taisho Toyama Pharmaceutical, and Torii Pharmaceutical. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Assessment of Antigenemia Assay for the Diagnosis of Cytomegalovirus Gastrointestinal Diseases in HIV-Infected Patients

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Abstract

We conducted a single-center prospective study to evaluate the utility of cytomegalovirus (CMV) antigenemia assay for the diagnosis of CMV-gastrointestinal disease (GID). The study subjects were HIV-infected patients with CD4 count $\leq 200\,\mu L$/cells who had undergone endoscopy. A definite diagnosis of CMV-GID was made by histological examination of endoscopic biopsied specimen. CMV antigenemia assay (C10/C11 monoclonal antibodies), CD4 count, HIV viral load, history of HAART, and gastrointestinal symptoms as measured by 7-point Likert scale, were assessed on the same day of endoscopy. One hundred cases were selected for analysis, which were derived from 110 cases assessed as at high-risk for CMV-GID after endoscopy screening of 423 patients. Twelve patients were diagnosed with CMV-GID. Among the gastrointestinal symptoms, mean bloody stool score was significantly higher in patients with CMV-GID than in those without (2.5 vs. 1.7, $p=0.02$). The area under the receiver-operating characteristic curve of antigenemia was 0.80 (95%CI 0.64–0.96). The sensitivity, specificity, positive likelihood ratio (LR), and negative LR of antigenemia were 75.0%, 79.5%, 3.7, and 0.31, respectively, when the cutoff value for antigenemia was ≥ 1 positive cell per 300,000 granulocytes, and 50%, 92.0%, 5.5, and 0.55, respectively, for ≥ 5 positive cells per 300,000 granulocytes. In conclusion, CMV antigenemia seems a useful diagnostic test for CMV-GID in patients with HIV infection. The use of ≥ 5 positive cells per 300,000 granulocytes as a cutoff value was associated with high specificity and high positive LR. Thus, a positive antigenemia assay with positive endoscopic findings should allow the diagnosis of CMV-GID without biopsy.

Introduction

CYTOMEGALOVIRUS (CMV) IS A MAJOR opportunistic pathogen of gastrointestinal diseases in patients with HIV infection. The incidence of CMV end-organ diseases, including CMV gastrointestinal disease (CMV-GID), has declined significantly following the introduction of highly active antiretroviral therapy (HAART). However, CMV-GID remains an important cause of morbidity and mortality because it can result in massive bleeding and gastrointestinal perforation.[1–5] Therefore, diagnosis at an early stage is essential.[6] Tissue biopsy is invasive and carries the risk of hemorrhage or perforation. Instead, endoscopy with biopsy provides definitive diagnosis.

The CMV blood antigenemia assay is a noninvasive method to detect CMV viremia and its utility has been evaluated previously for the diagnosis of CMV end-organ diseases in patients with HIV infection.[7–10] However, many of those studies included various types of CMV end-organ diseases such as CMV retinitis and pneumonia. To our knowledge, there are no studies that have investigated the value of

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CMV antigenemia assay in the diagnosis of CMV-GID, especially in HIV-infected patients.

We conducted a prospective study to assess the utility of the CMV antigenemia assay for the diagnosis of CMV-GID in patients with HIV infection.

Methods

Subjects

We prospectively recruited 423 HIV-infected patients who had undergone endoscopy between 2009 and 2012 at the National Center for Global Health and Medicine (NCGM), a 900-bed hospital located in the Tokyo metropolitan area and the largest referral center for HIV/AIDS in Japan. These patients were generally referred for endoscopy by the attending physician, based on the presence of gastrointestinal symptoms or for asymptomatic screening. Patients with CD4 count ≤200 were included in the analysis. We excluded patients who had received endoscopy for follow-up evaluation less than 3 months after treatment of gastrointestinal disease, who were under treatment for other CMV end-organ diseases, and those who were free of antigenemia.

The institutional review board of our hospital approved this study (approval No. 715).

Clinical factors

Gastrointestinal (GI) symptoms, CD4 count, HIV-RNA, history of HAART, and sexual behavior were collected before endoscopy. To evaluate GI symptoms, the modified gastrointestinal symptom rating scale (GSRS) rating on a 7-graded Likert scale was used.[11,12] The modified GSRS consists of the original GSRS (abdominal pain, heart burn, acid regurgitation, sucking sensation in the epigastrium, nausea and vomiting, borborygmi, abdominal distention, eructation, increased flatus, decreased passage of stools, loose stools, hard stools, urgent need for defecation, feeling of incomplete evacuation), plus odynophagia, chronic diarrhea, and bloody stool. Chronic diarrhea was defined as an episode lasting longer than 4 weeks.

Antigenemia assay

Antigenemia assay using C10/C11 monoclonal antibodies (Mitsubishi Chemical Medience, Tokyo, Japan) was performed as described previously.[13–15] A positive result of the CMV antigenemia assay was defined as ≥1 CMV-positive cell per 300,000 granulocytes applied. The assay was performed on the same day of endoscopy. For patients who were empirically prescribed anti-CMV therapy before endoscopy, CMV antigenemia obtained before initiating the therapy was used for analysis.

Diagnosis of CMV-GID

CMV-GID was suspected based on endoscopic findings, such as patchy erythema, edematous mucosa, multiple erosions, and ulcers.[16,17] Biopsy was performed when such endoscopic findings were encountered. CMV-GID was defined as the detection of large cells with intranuclear inclusions, alone, or in association with granular cytoplasmic inclusions on histological examination of biopsy specimens.[1] Biopsy sections were stained with hematoxylin and eosin, and also immunohistochemically stained with anti-CMV. The results were considered positive when the above-mentioned cells showed marked brown coloration in both nuclei and cytoplasm.

Statistical analysis

We divided patients into two groups based on the presence or absence of CMV-GID. Patient characteristics and clinical findings were then compared in the two groups using the Mann-Whitney U test, χ^2test, and Fisher's exact test for quantitative and qualitative variables, respectively. Area under the receiver-operating characteristic curve (ROC-AUC) analysis was used to quantify the accuracy of CMV antigenemia assay. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), positive likelihood ratio (LR), negative LR, and diagnostic odds ratio for the diagnosis of CMV-GID were also calculated for different cutoff values (≥1 positive cells per 300,000 granulocytes and ≥5 positive cells per 300,000 granulocytes). In a subgroup analysis stratified by patients with and without history of HAART, the sensitivity, specificity, PPV, and NPV were calculated using the cutoff value of CMV-positive cells of ≥1 per 300,000 granulocytes. All statistical analyses were performed using Stata software (version 10, Stata Co., USA).

Results

A total of 100 patients were selected for analysis after the application of the aforementioned exclusion criteria (Fig. 1). The majority of patients were males, and the median age was 40. The median CD4 count was 84 [interquartile range (IQR) 33.3–148.8] and 58.0% of the patients had history of HAART. Twelve patients were diagnosed with CMV-GID based on the abovementioned criteria (Fig. 2). In these patients, the median CMV antigenemia value was 4 positive cells per 300,000 granulocytes (range, 0–786). CMV-GID was localized to the upper GI tract in one patient, in the lower GI tract in 11, and in both parts in two.

Table 1 shows the baseline and demographic characteristics of the participating patients. Univariate analysis showed that significantly fewer patients with CMV-GID had history of HAART than those without CMV-GID ($p=0.016$) and median CD4 count was not significantly different between the two groups ($p=0.356$). The number of patients with positive CMV antigenemia was significantly higher in those with CMV-GID than those without ($p<0.01$). The mean bloody stool scores was significantly higher in patients with CMV-GID than in those without CMV-GID ($p=0.021$). In addition, there was a trend toward higher scores for heartburn ($p=0.064$) and chronic diarrhea ($p=0.078$) in patients with CMV-GID. The proportions of patients with the other symptoms were not different between the two groups.

ROC-AUC of the CMV antigenemia assay was 0.80 (95%CI 0.64–0.96). Table 2 lists the data that describe the diagnostic accuracy of CMV antigenemia assay. Using a cutoff value of ≥1 positive cell per 300,000 granulocytes for positive CMV antigenemia assay, the sensitivity, specificity, positive LR, and negative LR of antigenemia for CMV-GID were 75.0%, 79.5%, 3.7, and 0.31, respectively. The use of a cutoff value of ≥5 positive cells per 300,000 granulocytes yielded 50.0% sensitivity, 90.9% specificity, a positive LR of 5.5, and negative LR of 0.55 for the diagnosis of CMV-GID. Subgroup analysis

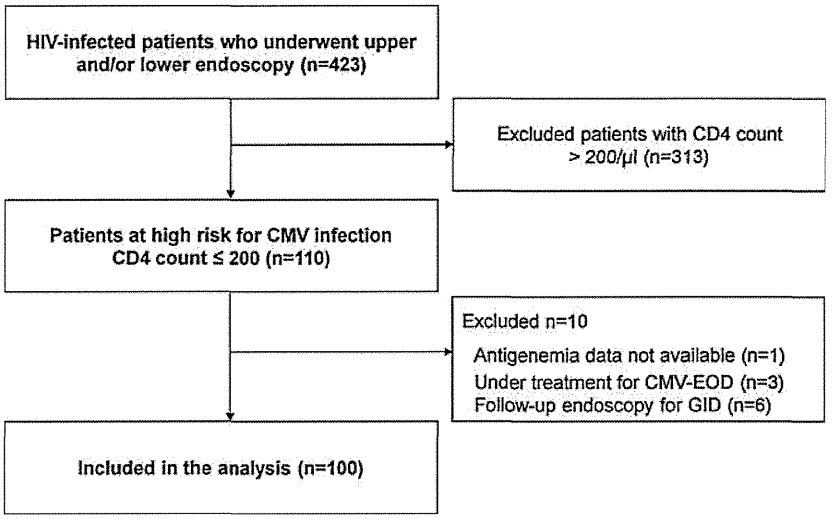

FIG. 1. Flow diagram of patient selection. CMV, cytomegalovirus; EOD, end-organ disease; GID, gastrointestinal disease.

showed a sensitivity of 66.7% and specificity of 83.6% for the assay for patients with history of HAART, while higher sensitivity (77.8%) and lower specificity (72.7%) were noted for those without ART.

Discussion

The present study provides the first prospective analysis of the CMV antigenemia assay in the diagnosis of CMV-GID in HIV-infected patients with 75.0% sensitivity and 79.5% specificity. The antigenemia assay is one of the most widely used methods for detecting reactivation of CMV infection, but only a few studies have examined its diagnostic value for CMV-GID,[18–21] and all were retrospective in design. Jang et al.[20] recently reported that the sensitivity and specificity of the CMV antigenemia assay for the diagnosis of CMV-GID were 54% and 88%, respectively, in patients with secondary immunodeficiency disease. Nagata et al.[21] also reported 65.4% sensitivity and 93.6% specificity of the CMV antigenemia assay for CMV-GID in patients with positive endoscopic findings. The present study demonstrated higher sensitivity (75.0%) and lower specificity (79.5%) than those studies. This difference in accuracy could be explained by the difference in the study population since only HIV-infected patients were included in our study, whereas previous studies included a substantial number of patients with immune deficiency due to etiologies other than HIV infection.

The sensitivity of antigenemia assay for the diagnosis of CMV end-organ disease in HIV-infected patients reported in previous studies was generally higher than that in the present

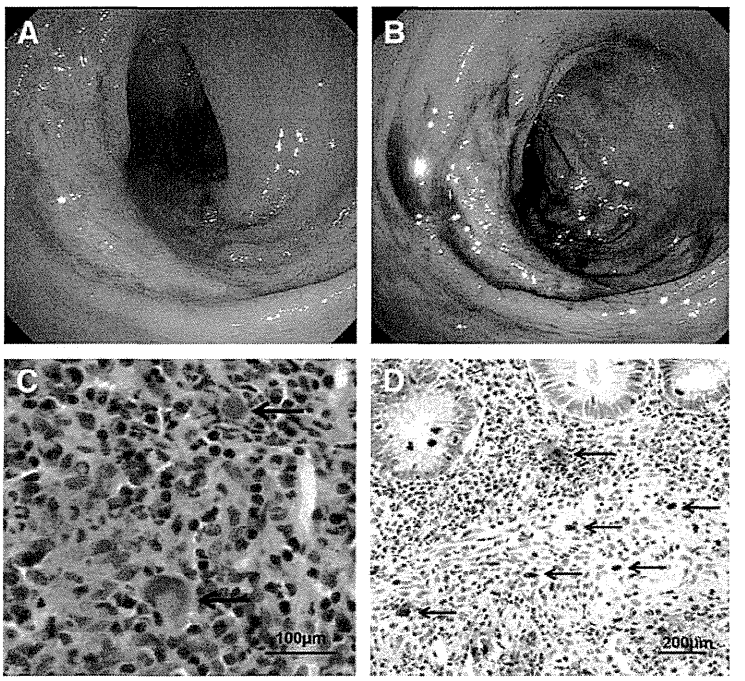

FIG. 2. Endoscopic and pathological features in representative cases. **(A)** Large distinct ulcer in the sigmoid colon; **(B)** Ulcer was more clearly observed with indigo carmine; **(C)** Large cells with intranuclear inclusions or associated with granular cytoplasmic inclusions (hematoxylin and eosin staining); **(D)** Cytomegalovirus (CMV)-infected cells (*arrows*) show *brown* coloration in both nuclei and cytoplasm (immunohistochemical staining with anti-CMV). (Color image can be found at www.liebertonline.com/apc.)