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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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

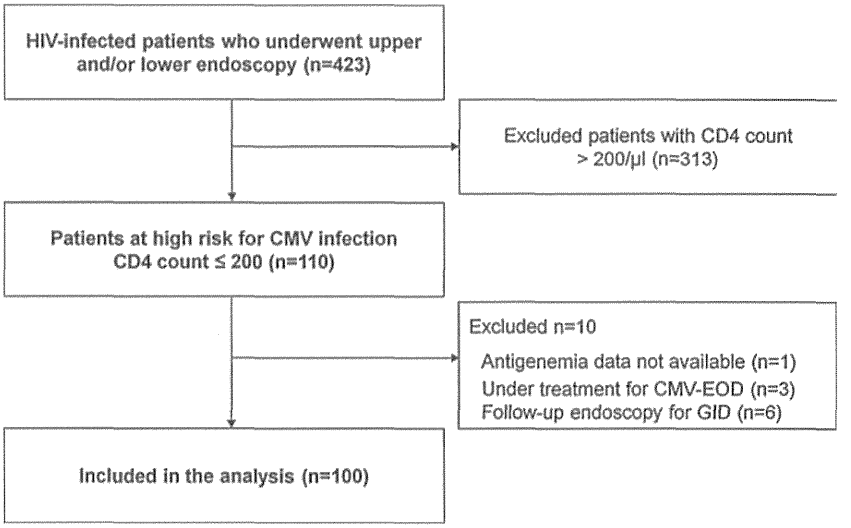

FIG. 1. Flow diagram of patient selection. CMV, cytomegalovirus; EOD, end-organ disease; GID, gastrointestinal disease.

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

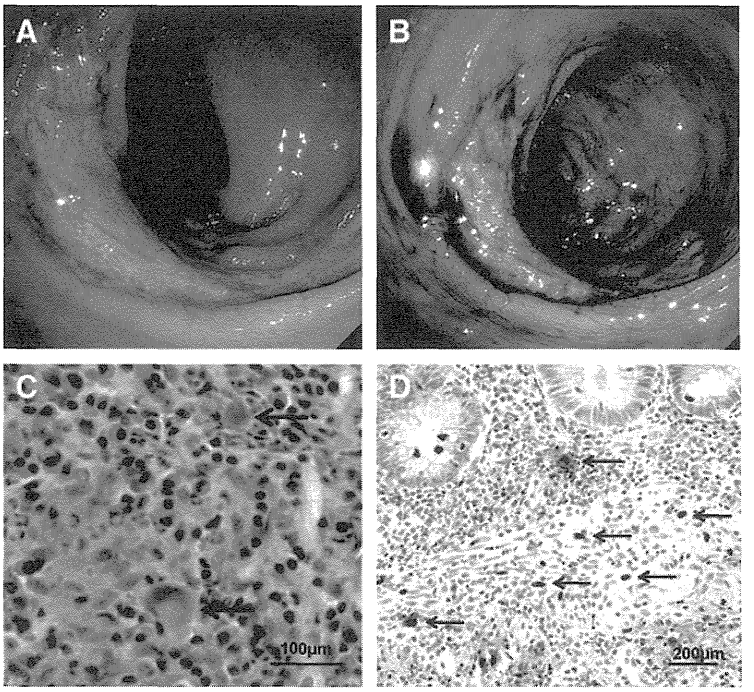

FIG. 2. Endoscopic and pathological features in representative cases. **(A)** Large distinct ulcer in the sigmoid colon; **(B)** Ulcer was more clearly observed with indigo carmine; **(C)** Large cells with intranuclear inclusions or associated with granular cytoplasmic inclusions (hematoxylin and eosin 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.)

Table 1. Clinical Characteristics of Patients With and Without CMV-GID

	CMV-GID (n=12)	*Non CMV-GID (n=88)*	p *Value*
Age (IQR)	39 (36–46)	40 (37–51)	0.451
Male gender (%)	11 (91.7)	87 (98.9)	0.227
CD4 count (/μL) (IQR)	68.5 (28.8–123.3)	84 (38.3–151.0)	0.356
HIV viral load (log10/mL) (IQR)	4.58 (3.27–5.24)	2.84 (1.60–5.08)	0.084
History of HARRT (%)	3 (5.2)	55 (62.5)	0.016
MSM (%)	9 (75.0)	69 (78.4)	0.723
Positive CMV antigenemia (%)	9 (75.0)	18 (20.5)	<0.001
Epigastric pain (SD)	2.5 (2.1)	1.8 (1.3)	0.373
Heartburn (SD)	2.5 (1.5)	1.8 (1.3)	0.064
Nausea and vomiting (SD)	2.4 (1.7)	2.0 (1.5)	0.384
Odynophagia (SD)	2.1 (1.7)	1.7 (1.5)	0.481
Chronic diarrhea (SD)	2.3 (1.3)	1.8 (1.4)	0.078
Bloody stool (SD)	2.5 (2.0)	1.7 (1.5)	0.021

CD4 cell counts within 1 week and HIV-RNA viral load within 1 month were checked at the day of endoscopy. A positive result for real-time HIV RNA was defined as ≥40 copies/mL. History of HAART was collected from the medical records prior to endoscopy. Sexual behavior was defined as men who have sex with men (MSM) or heterosexual.

CMV, cytomegalovirus; GID, gastrointestinal disease; HAART, highly active antiretroviral therapy; IQR, interquartile range; MSM, men who have sex with men; SD, standard deviation.

study.[8–10,22–26] This difference could be explained by the fact that the current study focused on gastrointestinal disease, while previous studies included various CMV diseases such as retinitis, cholangitis, pneumonia, and encephalitis.[8–10,22–26] The diagnostic accuracy of CMV antigenemia may vary depending on the site and extent of organ/tissue involvement.

Identification of CMV cells in tissue samples obtained by endoscopic biopsy is considered the gold standard for the diagnosis of CMV-GID.[1,2,6] The endoscopic findings in CMV-GID include ulcer and mucosal inflammation;[16,17] however, physicians may not consider it necessary to take a biopsy in patients with only mucosal inflammation without ulceration. Even in cases of severe deep or bleeding ulcers, some physicians may hesitate to perform a biopsy. In such cases, no definite diagnosis of CMV-GID can be made. Our results suggest that the CMV antigenemia assay is to some extent useful for the diagnosis of CMV-GID in patients with endoscopic findings, especially when CMV positive cell counts are high. Considering the high specificity and high positive LR (5.5) of the positive CMV cell count ≥5, the use of this method before endoscopy could potentially avoid complications due to biopsy.

One limitation of this study was the single-center nature of the investigation. Significant differences in independent factors were not detected in the present study probably due to the small number of patients with CMV-GID. For example, we used gastrointestinal symptoms with score of 7 points on the Likert scale, but the differences in most symptoms between patients with or without CMV-GID did not reach statistical significance due to the small number of cases. Further studies based on larger population are needed. Another limitation is a selection bias related to the selection criteria applied in the present study: only patients who underwent endoscopy for such reasons as symptoms and screening were included in the study.

In conclusion, the CMV antigenemia assay showed relatively good sensitivity and specificity for the diagnosis of CMV-GID in patients with HIV infection. Furthermore, specificity and positive LR improved when the cutoff value of CMV cell count was increased from 1 to ≥5 positive cells per 300,000 granulocytes. Considering the high specificity of the test, the use of this method before endoscopy could potentially avoid complications due to biopsy.

Table 2. Diagnostic Accuracy of CMV Antigenemia Assay for CMV-GID Using Different Cutoff Values and History of HAART

	Sensitivity (95%CI)	*Specificity (95%CI)*	*PPV (95%CI)*	*NPV (95%CI)*	*LR+ (95%CI)*	*LR− (95%CI)*	*OR (95%CI)*
CMV antigenemia ≥1 positive cell	75.0% (42.8–94.5)	79.5% (69.6–87.4)	33.3% (16.5–54.0)	95.9% (8.5–99.1)	3.7 (2.2–6.2)	0.31 (0.11–0.84)	11.7 (3.1–44)
CMV antigenemia ≥5 positive cells	50.0% (21.1–78.9)	90.9% (82.9–96.0)	42.9% (17.7–71.1)	93% (85.4–97.4)	5.5 (2.3–13.1)	0.55 (0.31–0.97)	10.0 (2.7–37.1)
History of HAART							
Yes[a]	66.7% (9.4–99.2)	83.6% (71.2–92.2)	18.2% (2.3–51.8)	97.9% (88.7–99.9)	3.7 (2.2–6.2)	0.31 (0.11–0.84)	10.2 (1.2–NA)
No[a]	77.8% (40.0–97.2)	72.7% (54.5–86.7)	43.8% (19.8–70.1)	92.3% (74.9–99.1)	2.9 (1.5–5.5)	0.31 (0.88–1.1)	9.33 (1.79–NA)

[a]Cutoff value of ≥1 positive cell per 300,000 granulocytes was used in the analysis.

CMV, cytomegalovirus; HAART, highly active antiretroviral therapy; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value.

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Author Disclosure Statement

The other authors declare no conflict of interest.

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Pharmacokinetics of Rifabutin in Japanese HIV-Infected Patients with or without Antiretroviral Therapy

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Abstract

Objective: Based on drug-drug interaction, dose reduction of rifabutin is recommended when co-administered with HIV protease inhibitors for human immunodeficiency virus (HIV)-associated mycobacterial infection. The aim of this study was to compare the pharmacokinetics of rifabutin administered at 300 mg/day alone to that at 150 mg every other day combined with lopinavir-ritonavir in Japanese patients with HIV/mycobacterium co-infection.

Methods: Plasma concentrations of rifabutin and its biologically active metabolite, 25-*O*-desacetyl rifabutin were measured in 16 cases with HIV-mycobacterial coinfection. Nine were treated with 300 mg/day rifabutin and 7 with 150 mg rifabutin every other day combined with lopinavir-ritonavir antiretroviral therapy (ART). Samples were collected at a median of 15 days (range, 5–63) of rifabutin use.

Results: The mean C_{max} and AUC_{0-24} of rifabutin in patients on rifabutin 150 mg every other day were 36% and 26% lower than on 300 mg/day rifabutin, while the mean C_{max} and AUC_{0-24} of 25-*O*-desacetyl rifabutin were 186% and 152% higher, respectively. The plasma concentrations of rifabutin plus its metabolite were similar between the groups within the first 24 hours, but it remained low during subsequent 24 to 48 hours under rifabutin 150 mg alternate day dosing.

Conclusion: Rifabutin dose of 150 mg every other day combined with lopinavir-ritonavir seems to be associated with lower exposure to rifabutin and its metabolite compared with rifabutin 300 mg/day alone in Japanese patients. Further studies are needed to establish the optimal rifabutin dose during ART. The results highlight the importance of monitoring rifabutin plasma concentration during ART.

Trial registration: UMIN-CTR (http://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=search&action=input&language=E) UMIN000001102

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Introduction

Rifabutin is commonly used for human immunodeficiency virus (HIV)-associated mycobacterial infections, especially during combination antiretroviral therapy (cART) containing HIV protease inhibitors (PIs), since it is less likely to induce hepatic microsomal enzymes than rifampicin [1–4]. Conversely, rifabutin and its active metabolite, 25-*O*-desacetyl rifabutin, are substrates of CYP 3A4 and concomitant use of PIs can elevate blood concentrations of rifabutin and 25-*O*-desacetyl rifabutin [3–8]. Such rise can increase the risk of side effects such as anterior uveitis [2,9–12]. Thus, a lower dose of rifabutin has been recommended in patients treated with PIs.

The previously recommended dose of rifabutin in combination with ritonavir-boosted PI (PI/r) [13] of 150 mg every other day, was associated with low rifabutin plasma concentrations and increases rate of acquired rifamycin resistance [14–17]. Furthermore, the Tuberculosis Trials Consortium (TBTC)/US Public Health Service Study 23 [14] suggested that AUC0–24 of 4.5 μg/mL is the cutoff value for risk of emergence of resistance to rifamycin. On the other hand, the combination of rifabutin at 150 mg thrice weekly with atazanavir-ritonavir provides exposure to rifabutin comparable to that of rifabutin 300 mg alone [11]. Thus, although 150 mg/day is the current recommended dose for rifabutin during PI/r-based cART [4], the optimal dose of rifabutin when used with a PI/r regimen remains to be established.

Ethnic differences, including body weight, renal clearance and various genetic factors like single nucleotide polymorphism (SNP), haplotype or DNA methylation [18,19], may alter the dose required to achieve a particular concentration of the drug in the circulation. Thus, pharmacokinetic studies involving different ethnic groups are needed to determine the recommended dose that take such factors into account. To our knowledge, there are no such pharmacokinetic studies for rifabutin use in Asians, who

are characterized by lower body weight compared with other ethnic groups. The present study was conducted to evaluate the pharmacokinetics of rifabutin in Japanese patients with HIV-1-related mycobacterial infection when used alone at 300 mg/day without cART and at 150 mg every other day when used in combination with lopinavir/ritonavir.

Methods

Ethics Statement

The study protocol was approved by the Ethics Committee of the National Center for Global Health and Medicine (NCGM-H20–580: approved on 7th February 2008). All participants provided their written informed consent before enrollment as indicated in the protocol.

The protocol for this study and supporting CONSORT checklist are available as supporting information; see File S1 for English translation of the protocol and File S2 for the Japanese original protocol and File S3 for CONSORT checklist.

Study design

Consecutive patients with HIV-1-related mycobacterial infection who received rifabutin-containing therapy at the National Center for Global Health and Medicine, Tokyo, Japan, between February 2008 and March 2009, were eligible for the study. After their written informed consent was provided, clinical history, physical examinations and laboratory tests (e.g., blood chemistry and complete blood cell count) were carried out within one week prior to the pharmacokinetic study. Patients were excluded if they were over 20 years of age or if they had abnormal liver function tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT) or total bilirubin (>3 times the upper limit of normal: ULN)], or severe renal dysfunction (creatinine clearance <30 ml/min), and in the case of female patients if they were pregnant or breastfeeding. Rifabutin was administered while fasting at 300 mg/day and the dose was adjusted when used with cART as recommended by the treatment guideline at the time of the study [13]. Medications administered concomitantly or within 2 weeks before the first study day were recorded. To evaluate the impact of rifabutin plasma concentration on treatment efficacy and adverse events, participants were followed up for at least 2 years after stopping rifabutin. Any side effect noted during rifabutin use or within four weeks after stopping rifabutin, its association with rifabutin was assessed.

Pharmacokinetic assays

Pharmacokinetic sampling commenced after 5 days of rifabutin-containing anti-mycobacterial therapy without (Group I) or with (Group II) cART. Sequential enrollment of a patient into both groups was accepted. Blood samples were collected just before rifabutin administration and then 0.5, 1, 2, 4, 6, 8 and 24 hours afterward. Patients of Group II treated with 150 mg of rifabutin every other day underwent additional sampling at 48 hours. The plasma concentrations of rifabutin and its major metabolite, 25-O-desacetyl rifabutin [20–23] were determined simultaneously by validated high-pressure liquid chromatography (HPLC). Blood samples were taken in heparin-containing tubes, placed on ice and centrifuged at 3000×g for 10 min. Then, the obtained plasma was deproteinized by using three times volume of methanol and centrifuged 15,000×g for 5 min, and the supernatant was used for assay. The HPLC standard for rifabutin and 25-O-desacetyl rifabutin were kindly provided by Pfizer Co. (Pfizer, Inc., NY). The HPLC system consisted of Agilent 1100 series (Agilent Technologies, Santa Clara, CA). Isocratic elution was performed using the Inertsil ODS-3 column (5 μm, 4.6 mm I.D. ×150 mm; GL Sciences Inc, Tokyo, Japan) with a guard column (5 μm, 4.6 mm I.D. ×10 mm; GL Sciences Inc). The UV detection wavelength was 280 nm. The mobile phase consisted of 9 mM

Table 1. Characteristics of study subjects.

	All (n = 16)	Group I (without cART, n = 9)	Group II (with cART, n = 7)	*p* value [a]
Male sex, n	16	9	7	
Age, median years (range)	36 (23–60)	36 (23–55)	35 (23–60)	0.53
Body weight, median kg (range)	57.3 (44–66)	58.0 (46–64)	56.5 (44–66)	0.98
Mycobacterium, multiple choice, n				
M. tuberculosis	13	7	6	1.00
M. avium	4	3	1	0.94
M. kansasii	1	0	1	0.85
CD4 count, median cells/mm^3 (range)	63 (2–164)	63 (2–164)	63 (19–135)	0.84
Plasma viral load, median log copies/ml (range)	4.97 (3.43–6.62)	4.98 (4.18–6.62)	4.95 (3.43–5.18)	0.10
AST, median IU/L (range)	29 (16–70)	25 (16–59)	30 (17–51)	0.65
ALT, median IU/L (range)	27 (13–70)	26 (23–70)	29 (19–70)	0.31
Time on rifabutin, median days (range)	15 (5–63)	7 (5–20)	29 (10–63)	0.017
Time on cART, median days (range)	14 (10–29)	–	14 (10–29)	–
Concomitant medications, n				
lopinavir-ritonavir	7	–	7	–
clarithromycin	3	2	1	1.00
fluconazole	1	0	1	0.85

[a]By Fisher's exact test for categorical data and Mann Whitney's U test for continuous variables.
cART, combination antiretroviral therapy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; IU, international unit.
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phosphate buffer (pH 6.8)-acetonitrile (30:70, v/v). The flow-rate was set at 1.0 ml/min and all separations were performed at 30°C in column oven.

Statistical and pharmacokinetic analyses

The area under the curve (AUC) was calculated using non-compartmental techniques (WinNonlin, ver. 5, Pharsight Corp., Mountain View, CA) based on the obtained values (AUC 0–24 h for all, AUC 0–48 h for Group II). The maximum plasma concentration (C_{max}) and time of C_{max} (T_{max}) were determined directly from the data.

Statistical analyses were performed using SPSS software package for Windows, version 17.0J (SPSS Japan Inc, Tokyo). Differences between groups were determined by using the Fisher's exact test for categorical data and the Mann Whitney's test for continuous variables. For all statistical analyses, differences were considered significant if the p value was less than 0.05.

Results

Patient characteristics

A total of 15 patients were enrolled in the study and 5 of 15 participated in both Group I and II. In total, twenty sampling was done for rifabutin pharmacokinetic analysis; 11 in Group I and 9 in Group II. Data from two sampling in Group I and 2 in Group II were excluded from the analysis because samples at 24-hour were unavailable or sampling was conducted earlier than 5 days of rifabutin use. As a result, data from 9 sampling in Group I and 7 sampling in Group II were used for analysis. The baseline characteristics of the 16 sampling cases are summarized in Table 1. All 7 patients of Group II were being treated with lopinavir/ritonavir as their cART, and thus rifabutin was administered at 150 mg every other day based on the guidelines at the time of the study [13]. Two cases of Group I and 1 of Group II were being treated with clarithromycin (CAM) [20] for systemic mycobacterial infection caused by *M. avium* or *M. intracullulare* (*M. avium* Complex: MAC). Five patients of Group I, in whom ART had been delayed several weeks after anti-mycobacterial therapy to prevent the immune reconstitution inflammatory syndrome (IRIS), were later enrolled in the study as patients of Group II (Figure 1). Accordingly, the median time of rifabutin use was longer in Group II than in Group I. There was no significant difference between the groups with regard to gender, age, body weight, CD4 counts, HIV-RNA load, type of mycobacteria and concomitant use of clarithromycin or fluconazole. All were Japanese and the median body weight was 57.3 kg. All patients completed their anti-

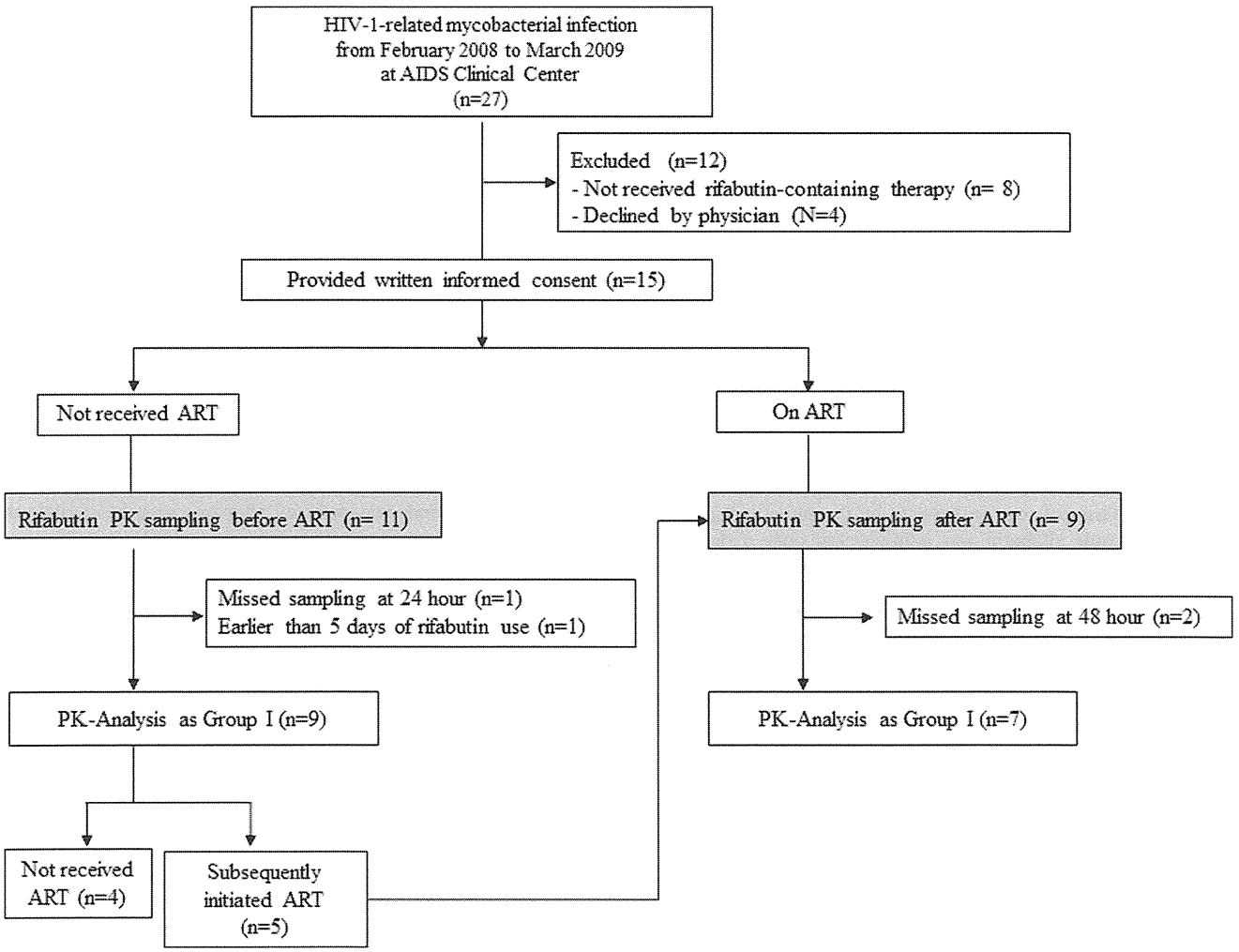

Figure 1. Flow chart of participants through the study. PK, pharmacokinetic; ART, antiretroviral therapy.
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Table 2. Pharmacokinetic parameters for rifabutin and 25-*O*-desacetyl rifabutin.

	Group I (without combination antiretroviral therapy, n = 9)				Group II (with combination antiretroviral therapy, n = 7)				*P* value[a]
	Median (range)		Mean (90% CI)		Median (range)		Mean (90% CI)		
Rifabutin									
C_{max} (μg/mL)	0.46	(0.15–0.86)	0.44	(0.39–0.49)	0.28	(0.10–0.44)	0.29	(0.25–0.33)	0.10
AUC_{0-24} (μg h/mL)	2.79	(1.32–15.7)	4.86	(3.83–5.90)	3.00	(1.13–5.43)	3.38	(2.92–3.84)	0.38
AUC_{0-48} (μg h/mL)[b]	5.59	(2.63–31.3)	9.71	(7.62–511.8)	4.21	(1.76–6.90)	4.58	(3.38–5.78)	0.32
T_{max} (h)	2.0	(2.0–4.0)	2.9	(2.6–3.1)	6.0	(2.0–12.0)	4.8	(4.1–5.1)	0.03
25-*O*-desacetyl rifabutin									
C_{max} (μg/mL)	0.00	(0.00–0.30)	0.05	(0.03–0.08)	0.13	(0.05–0.23)	0.14	(0.12–0.16)	0.05
AUC_{0-24} (μg h/mL)	0.00	(0.00–3.69)	0.82	(0.45–1.20)	1.52	(0.44–3.64)	2.07	(1.62–2.52)	0.12
AUC_{0-48} (μg h/mL)[b]	0.00	(0.00–7.38)	1.64	(0.89–2.39)	5.93	(0.44–7.21)	4.32	(3.27–5.38)	0.15
T_{max} (h)	6.0	(2.0–8.0)	5.3	(4.6–6.0)	6.0	(2.0–12.0)	5.7	(4.6–6.9)	0.87
Rifabutin plus 25-*O*-desacetyl rifabutin									
C_{max} (μg/mL)	0.47	(0.15–0.99)	0.49	(0.40–0.52)	0.42	(0.16–0.56)	0.39	(0.34–0.44)	0.54
AUC_{0-24} (μg h/mL)	3.36	(1.32–19.3)	5.49	(4.18–6.76)	6.23	(1.57–7.92)	5.27	(4.48–6.07)	0.93
AUC_{0-48} (μg h/mL)[b]	6.72	(2.63–38.7)	10.9	(8.35–13.5)	6.80	(2.20–14.1)	7.95	(6.40–9.49)	0.46

[a]By the Mann Whitney's *U* test.
[b]In Group I, AUC_{24-48} is assumed the same as AUC_{0-24} and AUC_{0-48} is calculated as double of AUC_{0-24} for comparison with Group II.
C_{max}, maximum plasma concentration; AUC, area under the curve; T_{max}, time of C_{max}; CI, confidence interval.
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mycobacterial treatment with clinical resolution of mycobacterial infections. None of the participants had treatment failure or relapse within more than 3 years of observation. Worsening of intra-abdominal lymphadenitis was observed in one patient with systemic M. avium infection at 8 months after stopping the 2-year rifabutin-containing anti-mycobacterial therapy, which excluded treatment failure or relapse. All patients confirmed complete adherence to anti-mycobacterial therapy and cART.

Pharmacokinetic parameters of rifabutin and its 25-*O*-desacetyl metabolite

The pharmacokinetic parameters of rifabutin and 25-*O*-desacetyl rifabutin are summarized in Table 2 and their mean plasma concentration-time data of 48 hours are illustrated in Figure 2A and 2B. For calculation of AUC_{0-48}, the data from 24 to 48 hours in Group I was assumed to be the same as that for 0–24 hours because rifabutin was administered once a day at the same dosage. As shown in Table 2, the mean values of C_{max} and AUC_{0-24} of rifabutin were 36% and 26% lower in Group II than in Group I, while the mean values of C_{max} and AUC_{0-24} of 25-*O*-desacetyl rifabutin were 186% and 152% higher in Group II than in Group I. However, the differences in the above values between the two groups were not statistically different. The low rifabutin concentration and high metabolite concentration in Group II may reflect the induction of rifabutin metabolism due to the longer duration of rifabutin use. Since 25-*O*-desacetyl rifabutin is microbiologically active against mycobacterium, total rifabutin activity might include rifabutin plus this metabolite. Figure 2C illustrates the mean plasma concentration of rifabutin plus the metabolite over time. Patients of Groups I and II had similar plasma concentrations of rifabutin plus the metabolite within the first 24 hours. However, the level of rifabutin plus the metabolite during the subsequent 24–48 hours was considerably lower in Group II than in Group I (dotted line in Figure 2C: Group I during 0–24 hours), whereas the AUC_{0-48} was not statistically different between the groups. Notably, 6 (67%) cases of Group I and 5 (71%) of Group II failed to achieve the AUC_{0-24} value suggested as risk for emergence of rifamycin-resistant *M. tuberculosis* [14] (4.5 μgh/mL). Neither C_{max} nor AUC_{0-24} of rifabutin and 25-*O*-desacetyl rifabutin were associated with age, body weight, body mass index, or CD4 count.

Rifabutin-associated side effects

Of the 15 participants, three patients developed side effects possibly related to rifabutin during the observational period; two of Group I developed skin rash and the other of Group II developed grade 2 rise in liver enzymes (ALT or AST 2.6–5.0 times of ULN). The skin rash appeared on day 11 of rifabutin-containing regimen in one patient and on day 28 in the other, and was resolved in both patients within several days after withdrawal of rifabutin. The rise in liver enzymes was detected after two months of rifabutin-containing regimen in combination with cART, and improved soon after discontinuation of rifabutin. Notably, the median CD4 counts in the three patients with rifabutin toxicity were significantly lower than in patients without rifabutin toxicity (12 *vs* 76, cells/mm^3, $p = 0.028$). However, rifabutin toxicity did not correlate with rifabutin AUC_{0-24}, C_{max}, or the concurrent use of cART (rifabutin AUC_{0-24}: $p = 0.37$, rifabutin C_{max}: $p = 0.86$, cART use: $p = 0.21$).

Discussion

In the present study, a low dose of rifabutin (150 mg every other day), in combination with lopinavir/ritonavir-containing cART, yielded comparable AUC_{0-24} of rifabutin and 25-*O*-desacetyl rifabutin to the commonly used dose of rifabutin of 300 mg/day. The advantage of the low-dose rifabutin included lower exposure to rifabutin and metabolite during the subsequent 24 to 48 hours in Japanese patients with HIV-mycobacteria co-infection. Since many participants started their cART after at least 1 month of

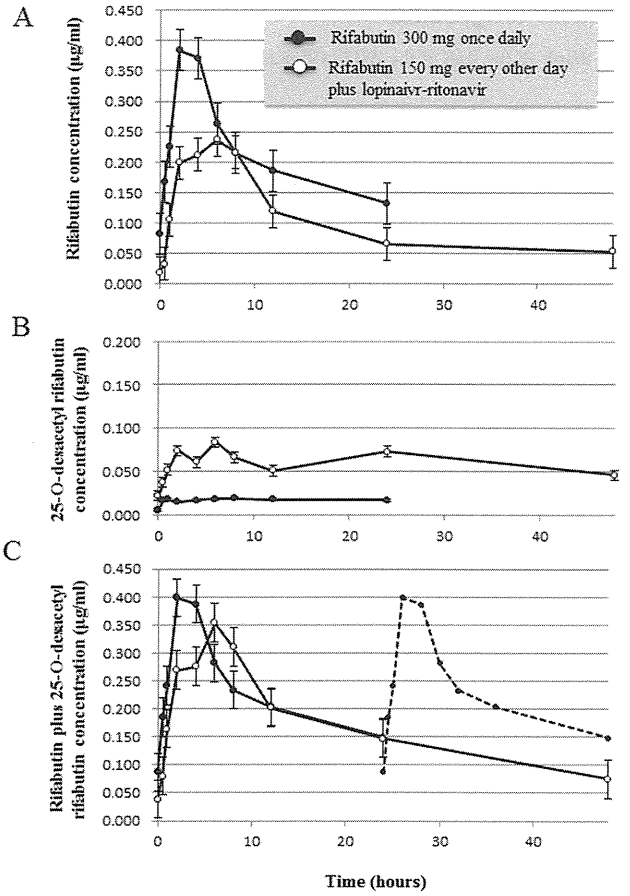

Figure 2. Mean plasma concentrations-versus-time plots of rifabutin (A), 25-*O*-desacetyl rifabutin (B), and rifabutin plus 25-*O*-desacetylrifabutin (C). Nine patients of Group I received 300 mg of rifabutin and 7 patients of Group II received 150 mg of rifabutin every other day with lopinavir/ritonavir-containing antiretroviral therapy. *Solid circles:* Group I, *open circles:* Group II. Data are mean ±1 standard errors. Dotted line in Figure C represents data of Group I during 0–24 hour for reference. RBT, rifabutin; PI/r, ritonavir-boosted protease inhibitor.
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anti-mycobacterial therapy in order to avoid deterioration by immune-reconstitution syndrome, the metabolism of rifabutin was induced upon the commencement of cART. This led to lower rifabutin concentration and higher 25-*O*-desacetyl rifabutin concentration in Group II but provided similar concentrations of rifabutin plus its active metabolite. However, on the day without medication, plasma concentrations of rifabutin and its active metabolite were lower in Group II, which were less than the susceptibility breakpoint level for *M. tuberculosis* proposed by others [20]. This suggests increased risk of emergence of rifamycin-resistant *M. tuberculosis* during the day without medication under low-dose rifabutin therapy, and that the currently recommended dosage 150 mg daily with PI/r is reasonable to this population as well. In this regard, Zhang et al. [11] reported that treatment with 150 mg/day rifabutin with atazanavir-ritonavir resulted in high risk of severe neutropenia. Furthermore, their post-hoc simulation showed that rifabutin 150 mg thrice weekly with atazanavir-ritonavir provided a comparable exposure to rifabutin compared with rifabutin 300 mg daily. Considering the risk of rifamycin-resistance and rifabutin toxicity, monitoring of rifabutin plasma concentration should be considered until the optimal rifabutin dosing during PI/r-based cART is fully established.

Although none of the patients showed treatment failure or relapse in this study, the rifabutin AUC0-24 observed in the study was in general close to the low end of the value reported in previous studies [7,14] and many participants [6 (67%) of Group I and 5 (71%) of Group II] failed to achieve AUC0-24 4.5 μgh/mL, the cutoff value suggested as risk for emergence of rifamycin-resistant M. tuberculosis [14]. One of the reasons for this discordant result might be the limitation of our study of small sample size involving several MAC and M. kansasii infections. Since acquisition of rifamycin-resistance M. tuberculosis was not frequent enough in this study group, it was difficult to evaluate the association with rifabutin pharmacokinetics and emergence of rifamycin-resistance. Other reasons may be the biological characteristics of rifabutin. Rifabutin has long postantibiotic effect against M. tuberculosis and MAC [20], shows extensive distribution in various tissues [21,22], and readily penetrates cell membranes of leucocytes [21,22]. These characteristics and their variations among patients can considerably influence the outcome of rifabutin-containing anti-mycobacterial therapy and therefore might be one of the explanations of favorable efficacy despite lower plasma concentrations of rifabutin in our study. Another limitation of this study is that plasma concentration of isoniazid was not measured, although low isoniazid plasma concentration is known to be independently related to treatment failure of HIV/TB co-infection [24]. Additionally, although there was no difference in rifabutin concentration among the patients with or without use of clarithromycin or fluconazole, those drugs can increase the rifabutin AUC and possibly affect the results. Since our study was enrolling patients with heterogeneous backgrounds in the real clinical setting, such as timing of sampling or different combination of anti-mycobacteial drugs, it was difficult to completely eliminate those impacts from the analysis. These conditions should be taken into account in the assessment of treatment outcome and associated factors in this study.

Among 15 study participants, 3 patients developed side effects related to rifabutin therapy, including skin rash and rise in liver enzymes. Notably, their CD4 counts were lower than those who did not show rifabutin toxicity, although rifabutin plasma concentrations and the concurrent use of cART were similar in the two groups. This is the first report implicating low CD4 count as a risk factor for rifabutin-related side effects. However, like other side effects of rifabutin, such as uveitis and leukocytopenia, which have been reported to be related to high-dose rifabutin or high rifabutin plasma concentrations [9–12], careful assessment involving larger population samples are needed to evaluate the association between high plasma concentrations of rifabutin and the related skin rash and hepatotoxicity.

In conclusion, in Japanese patients with HIV-mycobacteria co-infection, the plasma concentrations of rifabutin and active metabolite within the first 24 hours of treatment with low-dose rifabutin (150 mg every other day) combined with lopinavir-ritonavir, were similar to those encountered with 300 mg/day rifabutin alone. However, these concentrations decreased on the day without medication. Our findings could help determine the optimal dose of rifabutin during cART. Further studies are needed to establish the optimal dose of rifabutin during cART. Monitoring of rifabutin plasma concentration should be considered in patients with HIV-mycobacteria co-infection.

Supporting Information

Protocol S1 Summary in English. English translation of the protocol Summary.
(DOCX)

Protocol S1 Protocol and IC form in Japanese. The full version of the study protocol and the informed consent form in Japanese.
(PDF)

CONSORT Checklist S2.
(DOC)

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Author Contributions

Conceived and designed the experiments: JT. Performed the experiments: JT KS. Analyzed the data: JT. Contributed reagents/materials/analysis tools: JT KS. Wrote the paper: JT. Technical advice: YK. Patients' recruitment: KW TA HH HY K. Tsukada. Technical advice: K. Teruya HG SO.

PLOS ONE

Preemptive Therapy Prevents Cytomegalovirus End-Organ Disease in Treatment-Naïve Patients with Advanced HIV-1 Infection in the HAART Era

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Abstract

Background: The efficacy of preemptive therapy against cytomegalovirus (CMV) infection remains unknown in treatment-naïve patients with advanced HIV-1 infection in the HAART era.

Methods: The subjects of this single-center observation study were126 treatment-naïve HIV-1 infected patients with positive CMV viremia between January 1, 2000 and December 31, 2006. Inclusion criteria were age more than 17 years, CD4 count less than 100/μl, plasma CMV DNA positive, never having received antiretroviral therapy (ART) and no CMV end-organ disease (EOD) at first visit. The incidence of CMV-EOD was compared in patients with and without preemptive therapy against CMV-EOD. The effects of the CMV preemptive therapy were estimated in uni- and multivariate Cox hazards models.

Results: CMV-EOD was diagnosed in 30 of the 96 patients of the non-preemptive therapy group (31%, 230.3 per 1000 person-years), compared with 3 of the 30 patients of the preemptive therapy group (10%, 60.9 per 1000 person-years). Univariate (HR = 0.286; 95%CI, 0.087–0.939; p = 0.039) and multivariate (adjusted HR = 0.170; 95%CI, 0.049–0.602; p = 0.005) analyses confirmed that CMV-EOD is significantly prevented by CMV preemptive therapy. Multivariate analysis showed that plasma CMV DNA level correlated significantly with CMV-EOD (per log10/ml, adjusted HR = 1.941; 95%CI, 1.266–2.975; p = 0.002). Among the 30 patients on preemptive therapy, 7 (23.3%) developed grade 3–4 leukopenia. The mortality rate was not significantly different between the two groups (p = 0.193, Log-rank test).

Conclusions: The results indicate that preemptive therapy lowers the incidence of CMV-EOD by almost 25%. Preemptive therapy for treatment-naïve patients with CMV viremia is effective, although monitoring of potential treatment-related side effects is required.

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Introduction

Although the incidence of new cases of cytomegalovirus (CMV) end-organ disease (EOD) has decreased by 75%–80% with the advent of antiretroviral therapy (ART) and is currently estimated to be <6 cases per 100 person-years [1], CMV-EOD is still one of the major debilitating diseases among patients with advanced HIV infection.

CMV preemptive therapy is commonly used for patients scheduled for hematopoietic cell transplantation and solid organ transplantation, with clinical evidence of efficacy[2–6], however, it is not generally recommended in HIV patients [7] because of concerns regarding cost-effectiveness, risk of developing CMV resistance, side effect and the lack of a proven survival benefit [8]. A prospective trial in cooperation with Roche company to evaluate the efficacy of preemptive therapy in the pre-HAART (highly active ART) era showed significant preventive effect of oral ganciclovir (GCV) [9]. However; other studies conducted in both pre-HAART and HAART eras showed no significant effect [10,11]. However, the above studies included patients who had previously received ART. Therefore, the efficacy of preemptive therapy against CMV infection remains unknown in treatment-naïve patients with advanced HIV-1 infection in the HAART era.

We retrospectively compared the incidence of CMV-EOD in a cohort of ART-naïve adult patients with advanced HIV infection (low CD4 count and plasma CMV-DNA-positive). One group of these patients had received CMV preemptive therapy, while the other had not received such therapy.

Methods

Ethics Statement

The study was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine,

Tokyo. All patients included in this study provided a written informed consent for their clinical and laboratory data to be used and published for research purposes. This study has been conducted according to the principles expressed in the Declaration of Helsinki.

Study design

We performed a retrospective, single-center cohort study to elucidate the effectiveness of preemptive CMV treatment in HIV-infected patients with positive CMV viral load in the prevention of CMV-EOD. The study was conducted at the National Center for Global Health and Medicine, Tokyo, one of the largest clinics for patients with HIV infection in Japan, with more than 2,700 registered patients as of December 2006. The study population comprised treatment-naïve HIV infected patients aged more than 17 years, with CD4 count less than 100/μl and positive plasma CMV DNA viral load, who presented for the first time at our hospital between January 1, 2000 and December 31, 2006. Those with CMV-EOD at presentation and those with <3 months of follow-up were excluded. The follow-up period was 2 years from the initial visit.

Definition of CMV-EOD and CMV preemptive therapy

CMV-EOD was diagnosed according to standardized ACTG criteria (see Table S1) [11]. CMV retinitis was routinely screened for by dilated indirect ophthalmoscopy at both the first visit to the hospital and a few months after the commencement of ART. Other evaluations, such as endoscopy and bronchoscopy, were carried out in response to the symptoms and clinical condition. The diagnosis of CMV-EOD was established by at least two experts from our hospital.

CMV preemptive therapy was prescribed based on the clinician's assessment. CMV preemptive therapy was provided at our institution for patients with plasma CMV DNA of >5000 copies/ml. For patients with plasma CMV DNA of >3000 but less than 5000 copies/ml, the decision to initiate preemptive therapy was left to the attending physician, taking into consideration the overall clinical condition, such as subsequent rise in plasma CMV DNA and/or use of immunosuppressants, such as steroids and chemotherapeutic agents. Ganciclovir (GCV) and valganciclovir (VGCV) were the most commonly used agents, followed by foscarnet (FOS). The choice of induction (intravenous GCV 5 mg/kg every 12 hours, oral VGCV900 mg twice a day or intravenous FOS 90 mg/kg every 12 hours) or maintenance dose (intravenous GCV 5 mg/kg every 24 hours, oral VGCV 900 mg a day or intravenous FOS 90 mg/kg every 24 hours) was based on the clinical condition, such as the level of plasma CMV DNA or state of immunosuppression. The duration of therapy varied across individuals. CMV preemptive therapy was defined as at least a 7-day treatment with agents effective against CMV. The normal course of CMV preemptive therapy was 2 weeks of GCV induction dose followed by VGCV or GCV maintenance dose until plasma CMV DNA became negative. Patients were retreated based on clinicians' decision under some conditions with high risks for CMV-EOD as described above, if plasma CMV DNA became positive again after preemptive therapy.

Measurements

Plasma CMV DNA was measured using real-time PCR with a lower limit of detection of 200 copies/mL(CMV geniQ, Bio Medical Laboratory, Inc., Tokyo, Japan). Plasma CMV DNA was measured routinely at the first visit in patients with CD4 count of <100/μl, and re-examined every week or monthly, according to the level of plasma CMV DNA viral load or immune status and at the discretion of the attending physician.

In this study, the primary exposure variable was CMV preemptive therapy over no CMV preemptive therapy. The potential risk factors for CMV-EOD were determined based on previous studies [12–18], and included basic demographics and laboratory data, including age, sex, CD4 cell count, HIV viral load, plasma CMV DNA, and presence or absence of other medical conditions (concurrent use of steroids, concurrent chemotherapy and concurrent AIDS-defining diseases). For each patient, data on or closest to the day of the first visit to our hospital were retrieved for analysis.

Statistical analysis

Categorical and continuous baseline demographics and laboratory data were analyzed using Pearson's chi-square test and Student's t-test, respectively. The time from the first visit to our hospital to the development of CMV-EOD was analyzed by the Kaplan Meier method for patients on CMV preemptive therapy and no CMV preemptive therapy, and the log-rank test was used to determine the statistical significance. Censored cases represented those who died, dropped out, or were referred to other facilities before the end of follow-up period. The Cox proportional hazards regression analysis was used to estimate the impact of CMV preemptive therapy on the incidence of CMV-EOD. The impact of basic demographics, baseline laboratory data, and other medical conditions was also estimated with univariate Cox proportional hazards regression.

To estimate the unbiased prognostic impact of CMV preemptive therapy, we used three models based on multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for CMV preemptive therapy. Model 2 included age and sex, plus Model 1, in order to adjust for basic characteristics. In Model 3, we added variables with significant relation to CMV-EOD by univariate analysis or assumed as risk factor(s) for CMV-EOD in the literature[12–20] (e.g., CD4 count per 1/μl decrement, HIV viral load per log10/ml, CMVDNA viral load per log10/ml, concurrent steroid use, concurrent chemotherapy and concurrent AIDS defining disease). Statistical significance was set at two-sided p values <0.05. We used hazard ratios (HRs) and 95% confidence intervals (95%CIs) to estimate the impact of each variable on CMV-EOD. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Of the 199 HIV-infected patients with CD4 count <100/μl and positive plasma CMV DNA viral load referred to our hospital between January 1, 2000 and December 31, 2006, 126 patients were recruited in the study. Of these, 96 patients received CMV preemptive therapy while 30 did not (Figure 1). Table 1 lists the demographics, laboratory data, and medical conditions of the study population at baseline. The majority of the study population were males, East Asians, and relatively young (median: 42 years). There were no differences in baseline CD4 count (p = 0.595) and HIV viral load (p = 0.628) between the two groups. Patients of the CMV preemptive therapy group had higher plasma CMV DNA viral load (p<0.001), more likely to have developed AIDS defining diseases (p = 0.042), and tended to have been treated concurrently with steroids (p = 0.009), compared with the non-CMV preemptive group. There were no significant differences in the use of chemotherapy (p = 1.000) and in time to initiation of ART since study entry (p = 0.393, Table 1) between the two groups.

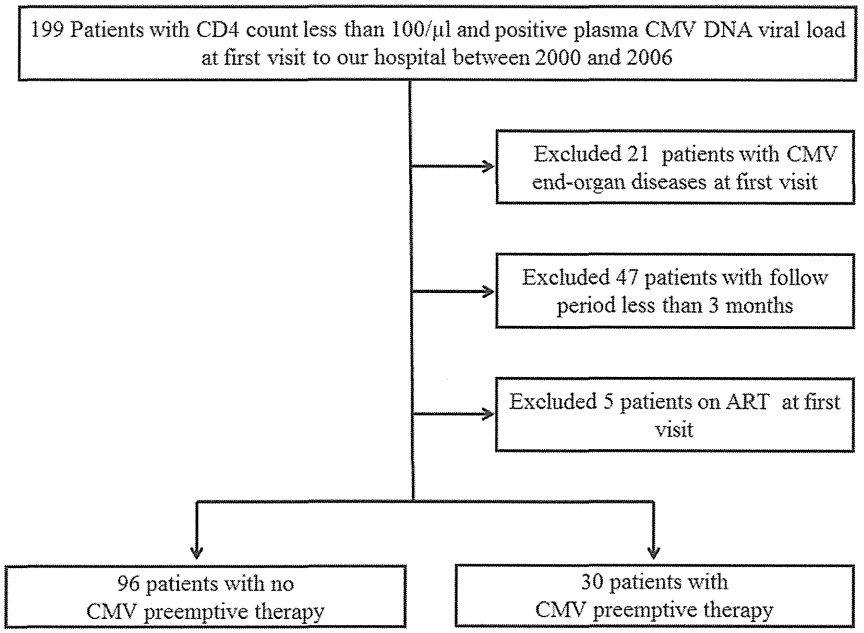

Figure 1. Flow chart of inclusion and exclusion criteria. Of the 199 subjects, 73 were excluded and the remaining 126 were included in the study. The latter group was divided into the preemptive therapy group (n = 30) and the non-therapy group (n = 96).
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During the follow-up period, CMV-EOD occurred in 3 (10.0%) patients of the preemptive therapy group and 30 (31.3%) of the non-preemptive therapy group, with an estimated incidence of 60.9 and 230.3 per 1000 person-years, respectively. Figure 2 depicts the time from the first visit to our hospital to the development of CMV-EOD by Kaplan Meier method in the two groups. The incidence of new cases of CMV-EOD was significantly higher in the non-preemptive therapy group, compared with the preemptive therapy group ($p = 0.027$, Log-rank test). The median time from the first visit to the diagnosis of CMV-EOD was 67 days (range, 25–67) for the preemptive therapy group, and 54 days (range, 14–326 days) for the non-preemptive therapy group.

Univariate analysis showed a significant relationship between CMV preemptive therapy and low incidence of CMV-EOD (HR = 0.286; 95%CI, 0.087–0.939; $p = 0.039$) (Table 2). On the other hand, high CMV viral load and HIV viral load tended to be associated with CMV-EOD, while old age, low baseline CD4

Table 1. Baseline demographics and laboratory data of patients who did and did not receive CMV preemptive therapy.

	Non-preemptive therapy (n = 96)	Preemptive therapy (n = 30)	P value
Sex (male), n (%)	88 (91.7)	29 (96.7)	0.685
Median (range) age	41 (24–76)	44 (25–66)	0.729
Ethnicity, n (%)			
East Asians	86 (89.5)	29 (96.7)	
Southeast Asian	5 (5.2)	0 (0.0)	
Black	3 (3.1)	0 (0.0)	
White	2 (2.1)	1 (3.3)	
Median (range) CD4 count (/μl)	28.0 (0–97)	35.5 (3–87)	0.595
Median (range) HIV RNA viral load (log10/ml)	5.3 (3–6)	5.35 (4–7)	0.628
Median (range) CMVDNA viral load (log10/ml)	3.0 (2–5)	4.3 (2–5)	<0.001
Concurrent AIDS, n (%)	78 (81.3)	29 (96.7)	0.042
Steroid use, n (%)	38 (39.6)	20 (66.7)	0.009
Chemotherapy, n (%)	9 (9.4)	2 (6.7)	1.000
Median (range) time (days) to ART*	66 (2–399)	59 (13–158)	0.393
Median (range) follow-up (days)	730 (14–730)	730 (25–730)	0.064

*11 missing values.
Categorical and continuous variables were analyzed using Pearson's chi-square test and Student's t-test, respectively.
doi:10.1371/journal.pone.0065348.t001

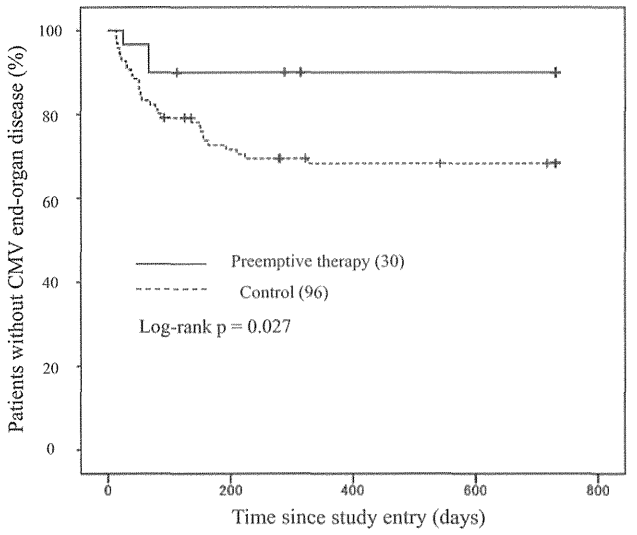

Figure 2. Kaplan-Meier curve showing the time to development of cytomegalovirus (CMV)- end-organ disease (EOD) in the preemptive and non-preemptive therapy groups. Compared to patients on CMV preemptive therapy, those who did not receive preemptive therapy were more likely to develop CMV-EOD (p = 0.027, Log-rank test).
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count, use of steroids, chemotherapy, and concurrent AIDS defining diseases were not associated with CMV-EOD. Multivariate analysis identified CMV preemptive therapy as a significant preventive factor against CMV-EOD after adjustment for age and sex (Model 2; adjusted HR = 0.289; 95%CI, 0.088–0.949; p = 0.041, Table 3), and after adjustment for other risk factors (Model 3; adjusted HR = 0.172; 95%CI, 0.049–0.602; p = 0.005, Table 3). In addition, multivariate analysis showed that high CMV viral load correlated significantly with CMV-EOD (Model 3; adjusted HR = 1.941; 95%CI, 1.266–2.975; p = 0.002, Table 3).

Of the 33 patients with CMV-EOD, 22 (66.7%) developed CMV retinitis, 4 (12.1%) developed esophagitis, 3 (9.1%) developed gastroduodenitis, 6 (18.2%) developed colitis and 1 (3.0%) developed pneumonitis. All 3 patients with CMV-EOD of the preemptive therapy group developed retinitis (Table 4).

Table 2. Results of univariate analysis to estimate the risk of various factors in inducing CMV end-organ disease.

	Hazard ratio	95% CI	P value
CMV preemptive therapy	0.286	0.087–0.939	0.039
Female	1.284	0.392–4.209	0.680
Age per 1 year	0.982	0.951–1.013	0.240
CD4 count per 1/μl decrement	1.001	0.989–1.013	0.867
HIV viral load per log10/ml	1.875	0.905–3.884	0.091
CMV viral load per log10/ml	1.450	0.984–2.136	0.060
Use of steroid	0.716	0.356–1.439	0.348
Chemotherapy	1.390	0.488–3.955	0.537
Concurrent AIDS	0.703	0.290–1.704	0.436

CI: confidence interval
The Cox proportional hazards regression analysis was used.
doi:10.1371/journal.pone.0065348.t002

Of 30 patients who received preemptive therapy, 20 (66.7%) received an induction dose of GCV, and 7 patients (23.3%) received maintenance dose. The remaining agents used for preemptive therapy were an induction dose of VGCV, a maintenance dose of FOS and an induction dose of cidofovir. The duration of the preemptive therapy varied between 7 days and 2 months. The following side effects were noted in patients on CMV preemptive therapy: grade 3/4 leukopenia (n = 7, 23.3%) and grade 2 hypercreatininemia (n = 1, 3.3%). Both side effects developed during the use of GCV. Five patients (5.2%) of the non-preemptive therapy group and 4 patients (13.3%) of the preemptive therapy group died during the study period. Of the former group, 3 deaths were due to opportunistic infections (cryptococcus meningitis, non-tuberculous mycobacterial infection and *Pneumocystis jiroveci* pneumonia), 1 due to bacterial infection (sepsis), and 1 due to suicide. Of the latter group, 2 deaths were due to opportunistic infections (malignant lymphoma and *P. jiroveci* pneumonia) and 2 due to bacterial infection (bacterial pneumonias). Deaths and bacterial infections related to preemptive therapy were not observed in our study. The mortality rate was not significantly different between the two groups (p = 0.193, Log-rank test, Figure 3).

Discussion

The results of this observational cohort of treatment-naïve HIV-infected patients with positive plasma CMV DNA showed a significantly lower incidence of CMV-EOD by one-fourths in the CMV preemptive therapy group than in the non-preemptive therapy group, over the 2-year observation period. This finding was significant, despite higher risk for CMV-EOD in the preemptive therapy group, such as higher plasma CMV DNA, higher prevalence of concurrent AIDS defining diseases and more concurrent steroid use, compared with the other group. Univariate and multivariate analyses identified anti-CMV preemptive therapy as a significant preventive factor against CMV-EOD.

Our study is the first to illustrate the significance of anti-CMV preemptive therapy in treatment- naïve HIV-infected patients with CMV viremia and CD4 count less than 100/μl in the HAART era. The hazard ratio of development of CMV-EOD decreased by 82.8% following preemptive therapy, compared with no preemptive therapy, even after adjustment for plasma CMV DNA viral load and other factors. The current guidelines do not generally recommend anti-CMV preemptive therapy although this is based on sparse evidence, such as cost effectiveness, CMV resistance, and drug side effects [7]. However, our study suggests that preemptive therapy is a feasible option, if the effective target of preemptive therapy could be selected. Furthermore, the study confirmed that plasma CMV DNA, a known risk factor for CMV-EOD [12–18], was a significant independent risk factor.

A few prospective clinical trials investigated the efficacy of preemptive therapy in both the pre-HAART era and HAART era. In these studies, oral GCV at 1000 mg thrice daily was used in the pre-HAART era regimen [9,10] while VGCV at 900 mg twice daily was the regimen used in the HAART era [11]. The patients investigated in the above three studies were HIV-treatment-experienced patients. One study in the pre-HAART era reported the efficacy of preemptive therapy in patients with CD4 count<50 μl [9], while the other studies showed no significant preventive effect [10,11]. In the ACTG A5030 study, the prospective clinical trial in the HAART era, which evaluated the efficacy of oral VGCV 900 mg twice a day for 3 weeks among HIV-infected patients with CD4 count <100 cells/mm^3, plasma HIV RNA >400 copies/mL, plasma CMV viremia and on stable

Table 3. Results of multivariate analysis to estimate the preventive effect of CMV preemptive therapy against CMV end-organ disease.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95% CI	HR	95%CI	HR	95%CI
CMV preemptive therapy*	0.286	0.087–0.939	0.289	0.088–0.949	0.172	0.049–0.602
Age			0.982	0.952–1.014	0.990	0.958–1.022
Female			1.033	0.310–3.441	0.988	0.267–3.653
CD4 count per 1/μl decrement					0.995	0.983–1.008
HIV viral load per log10/ml					2.217	0.912–5.393
CMV viral load per log10/ml*					1.941	1.266–2.975
Use of steroid					0.664	0.288–1.534
Chemotherapy					1.668	0.540–5.151
Concurrent AIDS					0.930	0.337–2.569

*P<0.05 in Model 3
HR: hazard ratio, CI: confidence interval
The Cox proportional hazards regression analysis was used.
Variables with significant difference by univariate analysis or assumed as risk factors for CMV-EOD in the literature were included in model 3.
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or no HAART, the authors reported a low incidence of CMV-EOD among subjects both with and without preemptive therapy [11]. The authors attributed the low incidence to improvement of immune function induced by potent ART. Actually, in that study [11], the number of patients who had received ART at study entry was about 80% of the total. In contrast, the subjects of our study were all treatment-naïve patients and possibly at higher risk for CMV-EOD compared to those enrolled in the ACTG A5030. Thus, the use of CMV preemptive therapy reported in our study under the clinical scenario of poor immune status without ART at study entry resulted in better outcome than in previous studies. In our study, there was no significant difference in the timing of ART between the two treatment groups. Although our study did not include the time to the initiation of ART as a variable in uni- and multivariate analysis because the values for 11 cases were missing, multivariate analysis with the time to the initiation of ART together with other variables similarly identified preemptive therapy as a significant preventive factor (adjusted HR = 0.235; 95%CI, 0.064–0.868; p = 0.030).

The survival benefits of CMV preemptive therapy were controversial in previous prospective clinical trials. One study suggested the survival benefits of 3 g/day oral GCV preemptive therapy [9], while other studies showed no evidence of the survival benefit [10]. On the other hand, two prospective cohort studies in the HAART era showed the relation between CMV viremia and high mortality [21] and suggested the benefit of CMV therapy [22], whereas our results showed no significant difference in mortality rate between the two groups. The reason for this discrepancy could be attributed to low mortality rate, small sample size and the disproportionally high risk of the therapy group in our study. The mortality rate (5.0 deaths per 100 person-years) in our study was similar to that in a study conducted in the HAART era (5.7 deaths per 100 person-years)[19] and was considerably lower than in studies from the pre-HAART era. Since the mortality rate has markedly decreased in advanced HIV infected patients following the introduction of potent ART in the HAART era [23,24], not only the survival benefit but also quality of life, such as improvement of eye function, should be emphasized in the future.

The side effects of preemptive therapy have also been of concern [25]. Our findings showed the development of grade 3 to 4 leukocytopenia in 23.3% of the patients who received intravenous GCV, and was the major side effect of preemptive therapy. Some patients who developed leukocytopenia required treatment with granulocyte colony-stimulating factor (G-CSF) and showed complete recovery. Thus; careful follow-up of patients on preemptive therapy is necessary. For these reasons, preemptive

Table 4. Details of CMV end-organ disease.

CMV-EOD	n (%)	Time to development (days)	Non-preemptive therapy group	Preemptive therapy group
Retinitis	22* (61.1%)	72 (14–326)	19* (57.6%)	3 (100%)
Esophagitis	4* (11.1%)	116.5(69–164)	4* (12.1%)	0
Gastroenteritis	3* (8.3%)	19 (14–40)	3* (9.1%)	0
Colitis	6* (16.7%)	40.5 (15–55)	6* (18.2%)	0
Pneumonitis	1 (2.8%)	31 (31–31)	1 (3.0%)	0
Total	36* (100%)	55 (14–326)	33* (100%)	3 (100%)

*Three patients of the non-preemptive therapy group had multiple CMV-EOD; one with retinitis plus esophagitis, one with retinitis plus gastroenteritis and the other with retinitis plus colitis.
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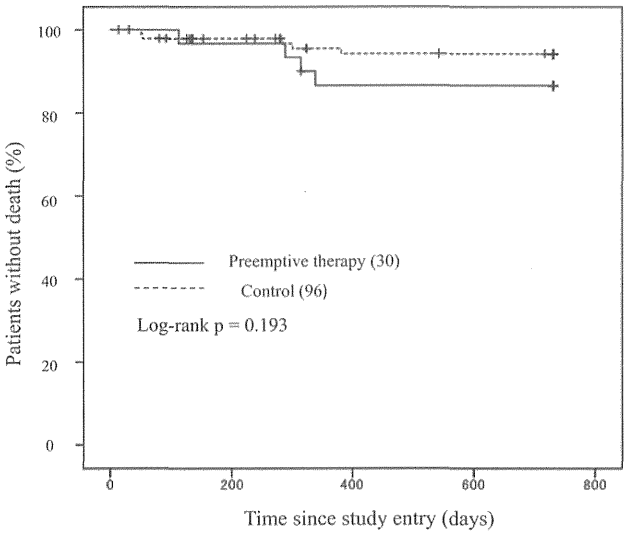

Figure 3. Kaplan-Meier curve showing the time to death in the preemptive and non-preemptive therapy groups. There was no significant difference in the survival rate between the two groups (p = 0.193, Log-rank test).
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therapy might place patients at greater risk in resource-limited setting, where close monitoring is difficult and the risk of bacterial infection is high. It is noteworthy, however, that death and bacterial infection related to preemptive therapy were not observed in our study.

The present study has several limitations. Due to its retrospective nature, it was not possible to control the baseline characteristics of the enrolled patients. However, patients with potential risk for CMV-EOD, such as those with high plasma CMV DNA, high concurrent AIDS and high steroid use, were more likely prescribed the preemptive therapy. It is noteworthy that the incidence of CMV-EOD was significantly lower in the preemptive therapy group despite this adverse environment.

Second, the criteria for treatment, choice of drugs and duration of CMV preemptive therapy were not rigidly controlled in the present study. Thus, it was difficult to determine which anti-CMV agent with what dosage is optimal for preemptive therapy. In the present study, about 90% of patients received induction dose or maintenance dose of GCV since the majority of patients of the preemptive therapy group were in-patients. Further prospective study is required to optimize effective preemptive therapy, including oral VGCV.

Third, CMV-EOD, especially enteritis, could have been overlooked at study entry since routine endoscopic screening was not performed, compared with screening for retinitis at the first visit. However, patients with abdominal pain were subjected to stool examination for occult blood, since the definition of CMV enteritis includes abdominal pain, and those with positive tests were subsequently considered for endoscopy. Thus, the possibility of latent CMV enteritis at study entry does not seem to have affected the results of the present study.

In conclusion, the present study demonstrated a lower incidence of CMV-EOD following CMV preemptive therapy by one-fourth, compared with no preemptive therapy, in treatment-naïve patients with CMV viremia. High plasma CMV DNA was identified as an independent risk for CMV-EOD. Further studies are warranted to elucidate the efficacy, safety and cost-effectiveness of anti-CMV preemptive therapy in HIV infected patients at high risk for EOD.

Supporting Information

Table S1 Definitions of CMV end-organ diseases used in this study.
(DOCX)

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Author Contributions

Conceived and designed the experiments: DM K. Tsukada K. Teruya. Performed the experiments: DM TN K. Teruya. Analyzed the data: DM HG YK SO. Contributed reagents/materials/analysis tools: YK K. Tsukada. Wrote the paper: DM TN HG SO.

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