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ORIGINAL ARTICLE

Prospective randomized comparison study of piperacillin/tazobactam and meropenem for healthcare-associated pneumonia in Japan

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Abstract Healthcare-associated pneumonia (HCAP) may have a more severe course than community-acquired pneumonia (CAP); hence, it is more likely to be caused by drug-resistant bacterial pathogens and anaerobes involved in aspiration pneumonia. We compared the efficacy and safety of initial empiric therapy with piperacillin/tazobactam (PIPC/TAZ, 13.5 g/day) with that of meropenem (MEPM, 1.5 g/day) as single broad-spectrum regimens with gram-negative and anaerobic coverage in patients with HCAP in Japan. The clinical cure rate was 75.9 % (22/29 cases) in the PIPC/TAZ group and 64.3 % (18/28 cases) in the MEPM group. The clinical efficacy rate was 87.9 % (29/33 cases) in the PIPC/TAZ group and 74.2 % (23/31 cases) in the MEPM group. The bacteriological eradication rate was 94.4 % (17/18) in the PIPC/TAZ group and 87.5 % (14/16) in the MEPM group. Adverse drug reactions were seen in 22.4 % (11/49 cases) of patients in the PIPC/TAZ group and 17.4 % (8/46 cases) of patients in the MEPM group. Although not statistically different, the PIPC/TAZ group had a slightly higher efficacy rate than the MEPM group. Both treatment regimens are tolerable and might be appropriate to use as initial empiric therapy for HCAP in Japan. To investigate the differences in efficacy profiles of those two regimens, a further confirmatory study with a larger cohort as determined by a power analysis is recommended.

Keywords Healthcare-associated pneumonia · Nursing and healthcare-associated pneumonia · Piperacillin/tazobactam · Meropenem · Antimicrobials

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Introduction

Pneumonia is the third leading cause of death in Japan, and mortality is especially high among elderly patients [1]. The Japanese Respiratory Society guidelines were established in August 2011 for the management of patients with nursing and healthcare-associated pneumonia (NHCAP) [2]. NHCAP differs from the healthcare-associated pneumonia (HCAP) that was described by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA); its definition was modified to fit the Japanese healthcare system [3]. This study was started in 2009, and the definition of NHCAP had not been established at that time. Hence, patients with HCAP were recruited in this study. In Japan, general hospitals have extended-care wards, and patients in these wards tend to stay in hospitals longer as compared to those in Western countries. Therefore, in this study, patients who resided in extended-care wards were included as HCAP cases.

HCAP may have a more severe course than community-acquired pneumonia (CAP) and is more likely to be caused

by drug-resistant bacterial pathogens and anaerobes involved in aspiration pneumonia [4–8]. Inappropriate therapy is a major risk factor for mortality and leads to extended hospital stay [9]. ATS/IDSA guidelines for nosocomial pneumonia recommend that all such patients receive empiric therapy with a multidrug regimen directed against drug-resistant organisms [3]. Nevertheless, Kett et al. [10] reported that compliance with ATS/IDSA guidelines for dual gram-negative coverage in patients who are at risk from multidrug-resistant pathogens was associated with increased mortality, which can be explained by antibiotic-specific toxic effects such as acute deterioration of renal function or neurotoxic effects. Brito and Niderman [11] developed an algorithm for empiric therapy of HCAP that suggests that not all such patients require a broad-spectrum multidrug regimen to achieve appropriate and effective therapy. Patients at risk for multidrug-resistant pathogens included those with severe illness or those with other risk factors including hospitalization in the past 90 days, antibiotic therapy in the past 6 months, poor functional status, and immune suppression.

For HCAP treatment, piperacillin/tazobactam hydrate (PIPC/TAZ) and carbapenems such as meropenem hydrate (MEPM) are recommended. However, limited data are available for comparing the effects of these two antibiotics against HCAP. In this study, the efficacy and safety of initial empiric therapy with PIPC/TAZ was compared to that with MEPM in patients with HCAP in Japan.

Patients and methods

Patients

We enrolled patients with HCAP from Nagasaki University Hospital and 14 affiliated facilities in Nagasaki Prefecture from October 1, 2009, to May 31, 2011. The study was conducted with prior approval from the ethics committee of each of the participating medical facilities and was registered on a clinical trial registry (UMIN ID No.: UMIN000002269). The study protocol was explained thoroughly to the patients or their legal representatives before the start of treatment, and written informed consent was obtained from each patient.

Patients with HCAP and a pneumonia severity index score [12] in risk class III or IV were required to fulfill all four of the following criteria: (1) appearance of new infiltrates on chest radiography or computed tomography; (2) either (a) resided in a nursing home, long-term care facility, or extended-care ward (for more than 48 h), (b) been hospitalized for $\geq$2 days in the past 90 days, (c) receiving outpatient intravenous therapy, or (d) receiving home wound care; (3) positive findings of at least one sign of inflammation such as white blood cell (WBC) count $>10{,}000/mm^3$ or $<4{,}500/mm^3$, increased C-reactive protein level, or fever $\geq$37 °C; and (4) positive findings of at least one of the clinical symptoms or signs, such as cough, purulent sputum, moist rales, dyspnea, and tachypnea.

The following participants were excluded: (1) patients with bronchial obstruction or history of obstructive pneumonia; (2) those unable to receive treatment every 8 h; (3) those with severe hepatic dysfunction or renal dysfunction (creatinine clearance $\leq$30 ml/min); (4) those for whom evaluation of clinical efficacy was difficult (including patients with cancer or other underlying diseases); (5) those infected with methicillin-resistant *Staphylococcus aureus* (MRSA), including suspected cases; (6) those receiving corticosteroids (prednisolone >10 mg/day); (7) those with a history of hypersensitivity to carbapenems, penicillins, or other beta-lactam antibiotics with or without beta-lactamase inhibitors; (8) those who were pregnant or lactating; (9) those with pneumonia severity index score in risk class V; and (10) those who were judged as otherwise ineligible by the attending physicians.

For the safety analysis, all randomized patients who received at least one dose of the study medication were included. Among the full analysis set (FAS), which included all subjects who received at least one dose of the study medication during this study and had a valid baseline and at least one post-baseline follow-up assessment of the primary outcome measure, all patients who completely met the inclusion and exclusion criteria with no protocol violations (per-protocol set, PPS) were included for efficacy analysis.

Study design, dosage, and administration method

This study was a multicenter, randomized, exploratory study. The patients were randomly allocated to receive either PIPC/TAZ (4.5 g) every 8 h or MEPM (0.5 g) every 8 h. Randomization by the minimization method was performed at a centralized website by attending physicians after obtaining written informed consent from each patient. Minimization factors included age and gender. The treatment period was 3–14 days in principle, but could be extended to a maximum of 21 days. Concomitant use of other antimicrobial agents was not allowed.

Evaluation

The primary endpoint of this study was clinical cure rate at the test-of-cure visit. Clinical cure was evaluated as (1) cure, which indicated continued improvement or complete resolution of the symptoms and no requirement for additional antimicrobial agents 7 days after the end of treatment (EOT); (2) failure, which indicated the treatment was

ineffective; or (3) indeterminate, which indicated evaluation of the clinical cure was enabled for any reason. The clinical cure rate was calculated among the evaluable patients; those who were evaluated as indeterminate were excluded.

The secondary endpoint of this study was clinical efficacy rate at EOT, which was determined by the evaluation committee based on the changes in the clinical symptoms, laboratory findings, and infiltrates on chest radiographs, by referring to the criteria of the Japan Society of Chemotherapy [13]. Clinical efficacy was evaluated as (1) effective based on improvement or complete resolution of symptoms, improvement in body temperature to 37 °C, chest radiograph score of ≤70 % of the previous value, WBC count <9,000/mm^3, and C-reactive protein count ≤30 % of the previous value; (2) ineffective based on no satisfaction of the efficacy standards; or (3) indeterminate because of inability to evaluate the clinical cure for any reason. The clinical efficacy rate was calculated among the evaluable patients; those who were evaluated as indeterminate were excluded.

The tertiary endpoints include bacteriological eradiation, survival, and safety parameters. Bacteriological eradiation was determined by the evaluation committee based on the criteria of the Japan Society of Chemotherapy [13] and evaluated as (1) eradication, which indicated absence of pathogen, (2) reduction, which indicated quantitative reduction of the original pathogen, (3) persistence, which indicated presence of the pathogen even after the complete course of antimicrobial agent therapy, (4) microbial substitution, which included appearance of new pathogens, or (5) indeterminate, which indicated inability to evaluate bacteriological eradiation for any reason. Survival was evaluated 30 days after EOT. For evaluation of safety parameters, all adverse events, including abnormal laboratory findings noted after the initiation of antibacterial agents, were recorded. An adverse event was considered to be an adverse drug reaction if a causal relationship with the antibacterial agent could not be ruled out; type of adverse event, and severity were recorded and evaluated for such events.

Statistical analysis

The data were analyzed using SAS version 9.2 (SAS Institute, Cary, NC, USA). P values <0.05 were considered statistically significant. The characteristics and underlying conditions of the patients between treatment groups were compared using the Fisher exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables. Fisher's exact test was performed to compare clinical cure rate and clinical efficacy rate in addition to the incidence of adverse drug reactions between treatment groups.

Results

Patients

A total of 95 patients were recruited from 15 facilities during the study period. A total of 49 and 46 patients were randomized to the PIPC/TAZ and MEPM treatment groups, respectively; 6 and 22 patients were excluded from the FAS and PPS, respectively. The trial profile is presented in Fig. 1. The characteristics and underlying conditions of the patients (PPS) are summarized in Table 1. The TAZ/PIPC and MEPM groups were well matched, and no significant differences were observed between the groups in the PPS.

Clinical efficacy

For clinical cure rate, 5 patients in the PIPC/TAZ group and 5 in the MEPM group were evaluated as indeterminate. Among those who were not evaluated as indeterminate, the clinical cure rate was 75.9 % (22/29 cases) in the PIPC/TAZ group and 64.3 % (18/28 cases) in the MEPM group; the rate did not differ significantly between the two groups. For clinical efficacy rate, 1 patient in the PIPC/TAZ group and 2 patients in the MEPM group were evaluated as indeterminate. Among those who were not evaluated as indeterminate, the clinical efficacy rate was 87.9 % (29/33 cases) and 74.2 % (23/31 cases) in the PIPC/TAZ group and MEPM group, respectively; the rate did not differ significantly between the two groups (Table 2).

Bacteriological efficacy

The most frequently isolated pathogen was *Streptococcus pneumoniae* (PIPC/TAZ, 5 cases; MEPM, 4 cases), followed by *Pseudomonas aeruginosa* (4 cases for each group), *Haemophilus influenzae* (PIPC/TA, 4 cases; MEPM, 2 cases), *Klebsiella pneumoniae* (PIPC/TAZ, 5 cases; MEPM, 1 case), methicillin-sensitive *S. aureus* (PIPC/TAZ, 3 cases; MEPM, 2 cases), and *Moraxella catarrhalis* (PIPC/TAZ, 2 cases; MEPM, 1 case). Polymicrobial infection was observed in 22.2 % of patients (8/36 cases). The eradication rates were 94.4 % (17/18) and 87.5 % (14/16) in the PIPC/TAZ and MEPM groups, respectively (Table 3).

Survival

Thirty days after EOT, overall survival rate of the PPS population was 93.7 % (excluding 4 cases unknown, 59/63 cases were still alive). Survival rate was 96.9 % in the PIPC/TAZ group (excluding 2 cases unknown, 31/32 cases were still alive) and 90.3 % in the MEPM group (excluding

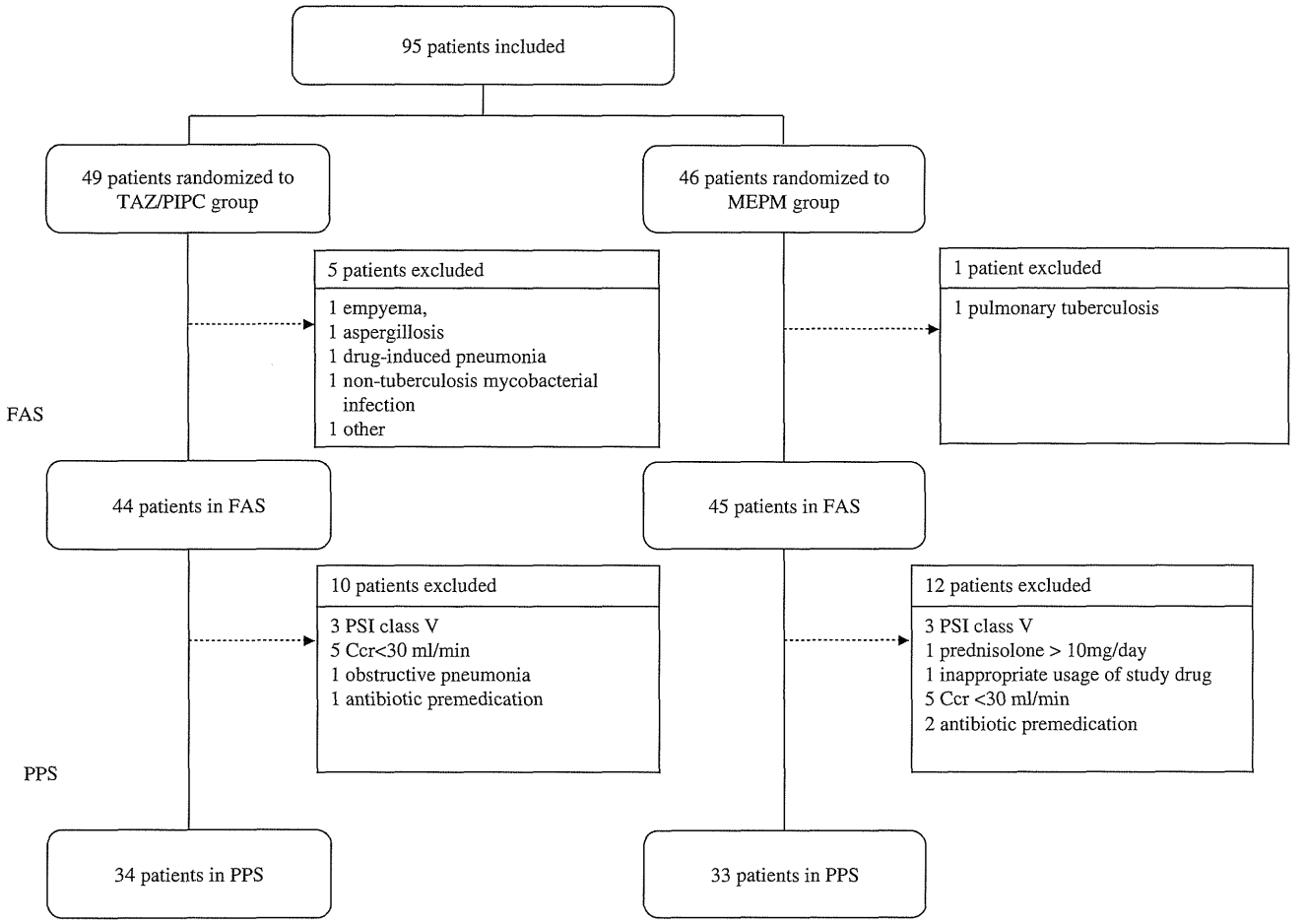

Fig. 1 Analysis sets investigated in this study. *TAZ/PIPC* piperacillin/tazobactam, *MEPM* meropenem, *FAS* full analysis set, *PPS* per-protocol set

2 cases unknown, 28/31 cases were still alive). Overall survival rate of the 12 cases who failed the treatment was 83.3 % (10/12 cases); 1 case died 1 day after EPT, and another death was confirmed 30 days after EOT.

Adverse drug reactions

In the PIPC/TAZ group, adverse drug reactions, such as diarrhea and hepatic dysfunction, were seen in 22.4 % of patients (11/49 cases). In the MEPM group, adverse drug reactions, seen in 17.4 % of patients (8/46 cases), included hepatic dysfunction. No significant differences were found between the two groups. All adverse drug reactions were mild or moderate in severity (Table 4).

Discussion

We designed a trial to compare two broad-spectrum single-agent regimens with gram-negative and anaerobic coverage: we compared the efficacy and safety of PIPC/TAZ with those of MEPM in patients with HCAP in Japan. The dosages of the drugs under study were 13.5 g/day (PIPC/TAZ) and 1.5 g/day (MEPM). MEPM was not approved for use at dosages of more than 1.5 g/day during the study period in Japan. Although not statistically different, the PIPC/TAZ group had a slightly higher efficacy rate than the MEPM group. Similarly, a previous study demonstrated a clinical efficacy rate of 83 % (62/75) for PIPC/TAZ that was used for the treatment of HAP patients [14]. Another study of PIPC/TAZ plus tobramycin versus ceftazidime plus tobramycin for HAP showed that PIPC/TAZ had a clinical efficacy rate of 74.4 % at 10–14 days after discontinuation of the study drugs [15]. In a study by Joshi et al. that compared PIPC/TAZ and imipenem/cilastatin, both in combination with tobramycin for HAP, PIPC/TAZ had a clinical cure rate of 68.4 % at 7–21 days after treatment [16]. The lower efficacy of MEPM may have been related to the low dose (1.5 g/day) but not to any background factors of the patients. As no renal dysfunction was reported in MEPM-treated patients (either in the PPS analysis or in the 5 MEPM-treated patients who were

Table 1 Characteristics of the patients

		Overall	Piperacillin/tazobactam (TAZ/PIPC)	Meropenem (MEPM)	*P* value
Number of patients		67	34	33	
Sex	Male/female	36/31	18/16	18/15	1.000[a]
Age (years)	≤59	6	3	3	0.5117[b]
	60–69	10	6	4	
	70–74	5	2	3	
	75–79	8	5	3	
	80–89	26	13	13	
	≥90	12	5	7	
Mean age (years)		78.3	77.6	79.1	
Weight (kg)	≤30	3	1	2	0.4502[b]
	30–40	20	7	13	
	40–50	27	19	8	
	≥50	17	7	10	
Mean weight		44.4	45.4	43.4	
PSI class	III	11	6	5	1.000[a]
	IV	56	28	28	
Mean PSI score		105	104	107	
Underlying disease, complication	+	66	33	33	1.000[a]
	–	1	1	0	
Treatment duration (days)	4–7	20	9	11	0.7665[b]
	8–14	40	22	18	
	15–21	7	3	4	
Mean treatment duration (days)		9.5	9.6	9.4	

[a] Fisher's exact test

[b] Wilcoxon rank-sum test

Table 2 The clinical cure and efficacy rate of TAZ/PIPC and MEPM

	TAZ/PIPC ($n = 34$)	MEPM ($n = 33$)	*P* value*
Clinical cure rate	75.9 % (22/29)	64.3 % (18/28)	0.395
Clinical efficacy rate	87.9 % (29/33)	74.2 % (23/31)	0.2076

For clinical cure rate, five patients in each of the PIPC/TAZ group and MEPM group who were evaluated as indeterminate were excluded. For clinical efficacy rate, one patient in the PIPC/TAZ group and two patients in the MEPM group who were evaluated as indeterminate were excluded

* Fisher's exact test

excluded from the PPS by low creatinine clearance rate), increasing the dosage to 3 g/day may be a potential treatment in HCAP. Although PIPC/TAZ at a dosage of 13.5 g/day showed a reasonable efficacy rate, it may be increased up to 18 g/day if needed. We observed diarrhea and hepatic dysfunction in the PIPC/TAZ group and hepatic dysfunction in the MEPM group; hence, caution is required when using higher dosages of these drugs.

Dysphagia caused by cerebrovascular diseases and disturbance of consciousness are well recognized in HCAP patients, and these are known to influence clinical outcomes [17, 18]. Risk factors of dysphagia such as neurological diseases, gastroesophageal diseases, presence of a feeding tube, and dementia were present in 65.7 % (44/67 cases) of patients in the PPS population. Additionally, because dysphagia may not be completely cured, aspiration pneumonia in such patients can frequently recur. Risk factors for dysphagia were observed in 87.5 % (7/8 cases) of patients who achieved clinical efficacy but not cure in our study. Along with antimicrobial treatment, evaluation of dysphagia and early initiation of rehabilitation in HCAP patients are important factors to consider.

Microbiological analyses revealed that the most frequent pathogen was *Streptococcus pneumoniae*, followed by *Psuedomonas aeruginosa*: both are major pathogens involved in CAP and HAP, respectively. Causative agents of HCAP varied, and included pathogens such as aerobic gram-positive cocci, including *S. pneumoniae* and *Staphylococcus aureus*, gram-negative bacilli, including *P. aeruginosa* and *Klebsiella pneumoniae*, and anaerobes. It is important to consider local patterns of microbiology, as each hospital or facility has unique bacteriology, and regimens of antimicrobial agents must be modified according to such local data [19]. *P. aeruginosa* and *K. pneumoniae* were frequently detected in our study. Therefore, PIPC/TAZ and

Table 3 Bacteriological efficacy

Causative organism	TAZ/PIPC						MEPM						*P* value*
	n	Eradication	Persisted	Substitution	Indeterminate	Eradication (%)	*n*	Eradication	Persisted	Substitution	Indeterminate	Eradication (%)	
Streptococcus pneumoniae	3	3	0	0	0	3/3	3	3	0	0	0	3/3	
Klebsiella pneumoniae	3	3	0	0	0	3/3	1	1	0	0	0	1/1	
Haemophilus influenzae	3	3	0	0	0	3/3	1	1	0	0	0	1/1	
Pseudomonas aeruginosa							3	1	1	1	0	2/3	
MSSA	1	1	0	0	0	1/1	2	1	0	1	0	2/2	
Moraxella catarrhalis	2	2	0	0	0	2/2							
Others	1	1	0	0	0	1/1	5	1	0	3	1	4/4	
S. pneumoniae + *H. influenzae*	1	1	0	0	0	1/1							
MSSA + *K. pneumoniae*	1	1	0	0	0	1/1							
MSSA + *P. aeruginosa*	1	1	0	0	0	1/1							
P. aeruginosa + *Escherichia coli*	1	0	1	0	0	0/1							
P. aeruginosa + *Enterococcus cloacae*	1	0	0	0	1								
P. aeruginosa + *H. influenzae*							1	0	1	0	0	0/1	
S. pneumoniae + *M. catarrhalis*							1	1	0	0	0	1/1	
PSSP + *K. pneumoniae* + *E. coli* + *P. aeruginosa*	1	0	0	1	0	1/1							
Total	19	16	1	1	1	17/18 (94.4)	17	9	2	5	1	14/16 (87.5)	0.5909

Eradication (%) = (eradication + substitution)/(total − indeterminate) × 100

MSSA methicillin-sensitive *Staphylococcus aureus*, *PSSP* penicillin-susceptible *Streptococcus pneumoniae*

* Fisher's exact test

Table 4 Adverse drug reactions

TAZ/PIPC			MEPM			*P* value*
22.4 % (11/49)			17.4 % (8/46)			0.613
Type	Severity	*n*	Type	Severity	*n*	
Diarrhea	Mild	2	Hepatic dysfunction	Mild	7	
Diarrhea	Moderate	1	Diarrhea	Mild	1	
Loose stool	Mild	1				
Hepatic dysfunction	Mild	2				
Hepatic dysfunction	Moderate	1				
Cardiomyopathy	Moderate	1				
Leukopenia	Mild	1				
Hematuria	Moderate	1				
Hyperkalemia	Mild	1				

* Fisher's exact test

MEPM were considered appropriate choices as empirical treatment, although there was no statistical difference in the bacteriological efficacy rate.

For safety profiles of the two regimens, adverse drug reactions were seen in 22.4 % of the PIPC/TAZ group and 17.4 % of the MEPM group; there was no statistically significant difference in the incidence rate between the two groups. In comparison, the incidence rate of adverse drug reactions of PIPC/TAZ (4.5 g 3–4 times daily) and MEPM (1 g 3 times daily) in clinical studies conducted in Japan were was 61.1 % (297/486 cases) [20] and 46.7 % (50/107 cases), respectively [21]. Thus, the incidence of adverse drug reactions of both PIPC/TAZ and MEPM were less in this study than in previously conducted clinical studies. Moreover, all adverse drug reactions seen in this study were mild or moderate in severity. Taken together, both treatment regimens are tolerable and might be appropriate to use as initial empiric therapy for HCAP in Japan. A further confirmatory study with a larger cohort as determined by a power analysis is recommended.

In conclusion, although not statistically different, the PIPC/TAZ group had a slightly higher efficacy rate than the MEPM group. Both treatment regimens are considered to be safe as initial empiric therapy for HCAP in Japanese patients. To investigate the differences in efficacy profiles of these two regimens, a further confirmatory study with a larger number of patients is necessary.

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Conflict of interest Shigeru Kohno has received honoraria, lecture fees, and research grants for activities other than this trial from Taisho-Toyama Pharma Co., Ltd. and Dainippon Sumitomo Pharma Co., Ltd. No other co-authors declare any conflicts of interest.

Appendix: Participating institutions and chief investigator of this study

Japanese Red Cross Nagasaki Genbaku Isahaya Hospital (Kiyoyasu Fukushima), Japanese Red Cross Nagasaki Genbaku Hospital (Koji Hashiguchi), Isahaya Health-Insurance General Hospital (Yuichi Inoue), Sasebo City General Hospital (Yuichi Fukuda), Senju Hospital (Hikaru Tanaka), Sasebo Chuo Hospital (Tsutomu Kobayashi), Hokusho Central Hospital (Yasuhito Higashiyama), National Hospital Organization Nagasaki Medical Center (Eisuke Sasaki), Nagasaki Municipal Hospital (Naofumi Suyama), Nagasaki Municipal Medical Center (Yoji Futsuki), Saiseikai Nagasaki Hospital (Keiko Iida), Izumikawa Hospital (Yoshihisa Kohno), Nagasaki Goto Chuoh Hospital (Hideki Ikeda), all in Nagasaki, and National Hospital Organization Ureshino Medical Center (Toyomitsu Sawai), Saga, Japan.

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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.

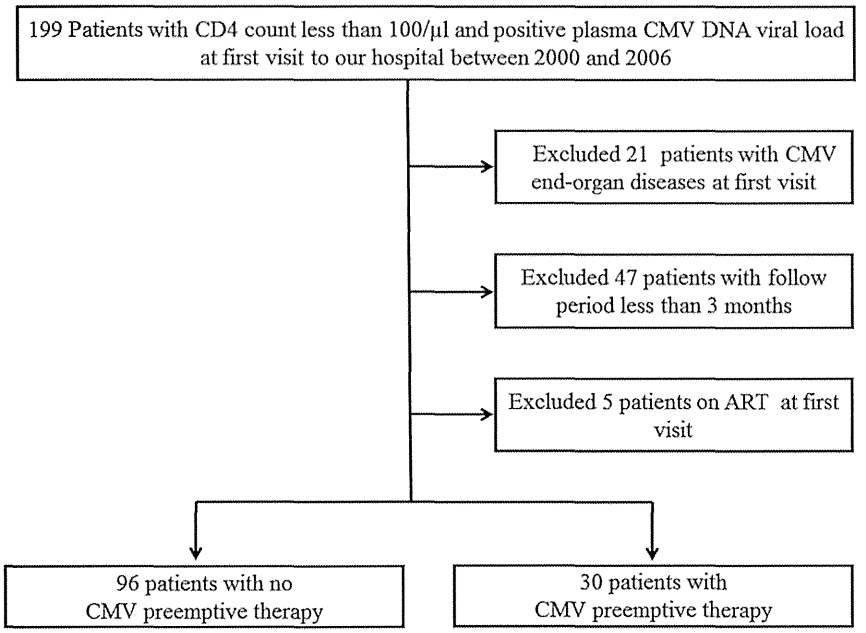

Figure 1. Flow chart of inclusion and exclusion criteria. Of the 199 subjects, 73 were excluded and the remaining 126 were included in the study. The latter group was divided into the preemptive therapy group (n = 30) and the non-therapy group (n = 96).
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During 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

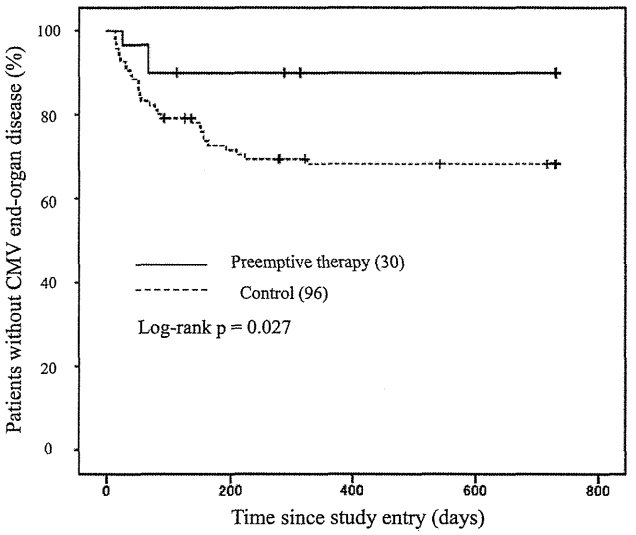

Figure 2. Kaplan-Meier curve showing the time to development of cytomegalovirus (CMV)- end-organ disease (EOD) in the preemptive and non-preemptive therapy groups. Compared to patients on CMV preemptive therapy, those who did not receive preemptive therapy were more likely to develop CMV-EOD (p = 0.027, 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.
doi:10.1371/journal.pone.0065348.t003

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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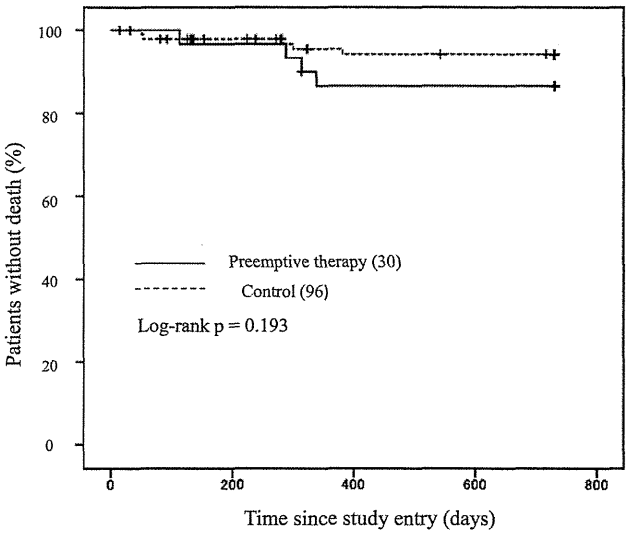

Figure 3. Kaplan-Meier curve showing the time to death in the preemptive and non-preemptive therapy groups. There was no significant difference in the survival rate between the two groups ($p = 0.193$, Log-rank test).
doi:10.1371/journal.pone.0065348.g003

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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MAJOR ARTICLE

Clinical Significance of High Anti-*Entamoeba histolytica* Antibody Titer in Asymptomatic HIV-1-infected Individuals

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Background. Anti-*Entamoeba histolytica* antibody (anti- *E. histolytica*) is widely used in seroprevalence studies though its clinical significance has not been assessed previously.

Methods. Anti-*E. histolytica* titer was measured at first visit to our clinic (baseline) in 1303 patients infected with human immunodeficiency virus type 1 (HIV-1). The time to diagnosis of invasive amebiasis was assessed by Kaplan-Meier method and risk factors for the development of invasive amebiasis were assessed by Cox proportional-hazards regression analysis. For patients who developed invasive amebiasis, anti-*E. histolytica* titers at onset were compared with those at baseline and after treatment.

Results. The anti-*E. histolytica* seroprevalence in the study population was 21.3% (277/1303). Eighteen patients developed invasive amebiasis during the treatment-free period among 1207 patients who had no history of previous treatment with nitroimidazole. Patients with high anti-*E. histolytica* titer at baseline developed invasive amebiasis more frequently than those with low anti-*E. histolytica* titer. Most cases of invasive amebiasis who had high anti-*E. histolytica* titer at baseline developed within 1 year. High anti-*E. histolytica* titer was the only independent predictor of future invasive amebiasis. Anti-*E. histolytica* titer was elevated at the onset of invasive amebiasis in patients with low anti-*E. histolytica* titer at baseline.

Conclusions. Asymptomatic HIV-1-infected individuals with high anti-*E. histolytica* titer are at risk of invasive amebiasis probably due to exacerbation of subclinical amebiasis.

Keywords. seroprevalence; *Entamoeba histolytica*; HIV-1; anti-*E. histolytica* antibody; amebiasis.

Invasive amebiasis caused by *Entamoeba histolytica* is the second most common cause of parasite infection-related mortality worldwide, accounting for 40 000–100 000 deaths annually [1]. Recently, it was reported that invasive amebiasis is prevalent not only in developing countries where food or water is contaminated with stool, but also in East Asian developed countries (Korea, China, Taiwan and Japan) and Australia as a sexually transmitted infection (STI) [2–4]. On the other hand, the annual incidence of human immunodeficiency virus type 1 (HIV-1) infection is also on the rise among men who have sex with men (MSM) in these countries [5–8], with resultant growing concern regarding invasive amebiasis in HIV-1-infected MSM [9–14].

Serum anti-*E. histolytica* antibody (anti-*E. Histolytica*) is widely used as an index marker for the presence of amebiasis. It is used not only in developing countries [15–22] but also in developed countries where amebiasis is spreading as an STI [3, 9, 23–26]. Furthermore, the seroprevalence of anti-*E. histolytica* antibody in HIV-1-infected individuals is generally higher than in HIV-1 negative ones [3, 9, 15, 24]. However, only limited information is available on the seroprevalence of amebiasis in Japan [25, 26] despite the increasing number of invasive amebiasis among HIV-1-infected individuals reported lately [27, 28].

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Serum anti-*E. histolytica* antibody is also widely used for the diagnosis of invasive amebiasis based on the high sensitivity and good differentiation ability from other amoeba species, such as *Entamoeba dispar* and *Entamoeba moshkovskii* [29]. However, the primary disadvantage of this method is that it cannot distinguish current infection from past infection. Moreover, anti-*E. histolytica* antibody titer can be elevated even in asymptomatic infected individuals, and seroconversion of anti-*E. histolytica* was reported in the absence of any symptoms in longitudinal follow-up in endemic areas [14]. At present, the pathogenesis of amebiasis in asymptomatic anti-*E. histolytica* -positive individuals remains poorly understood.

In the present study, we found high seroprevalence of anti-*E. histolytica* antibody in HIV-1-infected adult Japanese. Retrospective analysis of these seropositive individuals indicated that those with high anti-*E. histolytica* titer are prone to future invasive amebiasis. These findings highlight the clinical significance of anti-*E. histolytica* positivity and enhance our understanding of the pathogenesis of invasive amebiasis.

MATERIALS AND METHODS

Ethics Statement

This study was approved by the Human Research Ethics Committee of our hospital, the National Center for Global Health and Medicine, Tokyo. The study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

Study Design and Population

The present study was a single-center retrospective cohort study. Our facility is one of the largest core hospitals for patients with HIV-1 infection in Japan, with >3000 registered patients. The study population was HIV-1-infected patients who were referred to our hospital for management of HIV-1 infection for the first time between January 2006 and April 2012.

Anti-*E. histolytica* Antibody Testing

Indirect fluorescent-antibody (IFA) assay was used for the detection of anti-*E. histolytica* antibody in serum by using a slide precoated with fixed *E. histolytica*. This method can distinguish amebiasis caused by *E. histolytica* from that caused by other amoeba species, such as *E. dispar* and *E. moshkovskii*. The sensitivity and specificity of this method for the detection of *E. histolytica* infection are comparable with other methods, such as counterimmunoelectrophoresis and indirect hemagglutination amebic serology [29, 30]. The commercial kit, Amoeba-Spot IF (bioMerieux SA), is currently approved for the diagnosis of *E. histolytica* infection in Japan. Based on the instructions enclosed with the kit, the biological samples were initially diluted at 1:100 with phosphate-buffered saline (PBS) and then incubated for 30 minutes at room temperature on slides precoated with fixed *E. histolytica*. Then, the slides were washed with PBS twice, treated with the fluorescent-labeled anti-human antibodies, and incubated for another 30 minutes at room temperature. The slides were washed again, and cover slips with buffered glycerol were placed over the slides. Fluorescence in each slide was examined with fluorescence microscope and compared with negative control slides. Seropositivity was defined as positive response in serum sample diluted at 1:100, and anti-*E. histolytica* titer was determined by the highest dilution for the positive response.

Development of Invasive Amebiasis in Patients Without History of Nitroimidazole Treatment

Newly registered HIV-1-infected individuals who underwent anti-*E. histolytica* testing at first visit were included in this analysis. Patients were excluded from the follow-up study (1) if they had been treated previously with nitroimidazole (metronidazole or tinidazole) or (2) if they were treated with nitroimidazole at first visit to the clinic. The clinical characteristics and results of serological tests for other STIs, such as syphilis and hepatitis B and C viruses (HBV and HCV), were collected from the medical records. The follow-up period spanned from the time of the first visit to May 2012, unless patients died from other causes during this period, dropped out, or were referred to other facilities.

The diagnosis of invasive amebiasis was based on the medical records of 3 different clinicians and satisfied one of the following 2 criteria, as described elsewhere [12–14] ; (1) identification of erythrophagocytic trophozoites in biological specimens (stool or biopsy sample) of HIV-1-infected patients with symptoms of invasive amebiasis, such as fever, tenesmus, and diarrhea, (2) identification of liver abscess by imaging studies in seropositive (titer $\geq$×100) patients with symptoms related to invasive amebiasis who showed clinical improvement after nitroimidazole monotherapy. For patients who developed invasive amebiasis during follow-up, we compared anti-*E. histolytica* titer at the time of onset of invasive amebiasis with those at first visit (baseline) and after nitroimidazole therapy.

Statistical Analysis

The patients' characteristics and results of serological tests on STIs were compared using χ^2 test or Student *t* test for qualitative or quantitative variables, respectively. The time to the diagnosis of invasive amebiasis was calculated from the date of the first visit of our hospital to the date of diagnosis of invasive amebiasis. Censored cases represented those who died, dropped out, or were referred to other facilities during the follow-up. The time from first visit to the diagnosis of invasive amebiasis was calculated by the Kaplan-Meier method followed by log-rank test to determine the statistical significance. The Cox proportional-hazards regression analysis was used to estimate the impact of anti-*E. histolytica* titer at baseline on the incidence of invasive amebiasis. The impact of basic clinical characteristics,

Table 1. Characteristics of All Patients Who Underwent Anti-*E. histolytica* Testing (n = 1303)

	Anti-*E. histolytica* Negatives (n = 1026)	Anti-*E. histolytica* Positives (n = 277)	*P* Value
Age,years (range)	36 (18–77)	37 (19–74)	.06
Japanese nationality, no. (%)	921 (89.8%)	250 (90.3%)	.81
Male sex, no. (%)	960 (93.6%)	272 (98.2%)	.003
MSM, no. (%)	789 (76.9%)	245 (88.4%)	<.001
TPHA test positive, no. (%)	366/1012 (36.2%)	151/275 (54.9%)	<.001
HBV exposure,[a] no. (%)	524/1017 (51.5%)	187/272 (68.8%)	<.001
HCV Ab positive, no. (%)	40/1011 (4.0%)	5/273 (1.8%)	.09
Past history of IA, no. (%)	13 (1.3%)	60 (21.7%)	<.001
Diagnosis of IA at first visit, no. (%)	1 (0.1%)	7 (2.5%)	<.001

Abbreviations: Ab, antibody; Anti-*E. histolytica*, anti *Entamoeba histolytica* antibody; HBV, hepatitis B virus; HCV, hepatitis C virus; IA, invasive amebiasis; MSM, men who have sex with men; TPHA, *Treponema pallidum* hemagglutination.

[a] HBV exposure: HBsAg-positive or HBsAb-positive, and/or HBc-Ab positive.

such as sexuality and serology status of other STIs, was estimated with univariate Cox proportional hazards regression. We also conducted multivariate Cox hazards regression analysis using variables identified in univariate analysis with *P* values of < .20. In all analyses, statistical significance was defined as 2-sided *P* value of < .05. We used the hazard ratio (HR) and 95% confidence interval (95%CI) to estimate the impact of each variable on the development of invasive amebiasis. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL).

RESULTS

Clinical Characteristics of Asymptomatic Anti-*E. histolytica*-positive HIV-1-infected Patients

A total of 1519 patients were referred to our hospital during the study period. Anti-*E. histolytica* testing was conducted in 1303 patients at first visit, including 73 with history of invasive amebiasis, and anti-*E. histolytica* was positive in 277 of these (21.3%). Among the anti-*E. histolytica*-positive individuals, the rates of MSM (88.4%) and those with previous exposure to syphilis (TPHA test positive) (54.9%) and HBV (68.8%) were higher than those of anti-*E. histolytica*-negatives individuals, indicating that sexually active MSM are prone to *E. histolytica* infection among HIV-1-infected individuals in Japan (Table 1). Eight patients were diagnosed with invasive amebiasis at first visit, including 7 cases of amebic colitis and 1 case of amebic liver abscess, and they were treated immediately with metronidazole.

Incidence of Invasive Amebiasis During Follow-up of HIV-1 Infected Individuals

To assess the frequency of development of invasive amebiasis in patients free of symptomatic invasive amebiasis and who had not previously received nitroimidazole therapy, we excluded 96 patients from the analysis, including 73 patients because they had been treated previously for invasive amebiasis, and 23 patients (7 cases of amebic colitis, 1 case of amebic liver abscess, and 15 asymptomatic but anti-*E. histolytica*-positive cases treated preemptively) because they were treated with nitroimidazole at first visit (Figure 1). The remaining 1207 patients, including 195 anti-*E. histolytica*-positive patients (16.2%), were followed-up for median period of 25.3 months (interquartile range: 7.0–47.2). During the follow-up period, 18 patients developed invasive amebiasis (median time to onset: 9.1 months), including amebic appendicitis in 1 patient

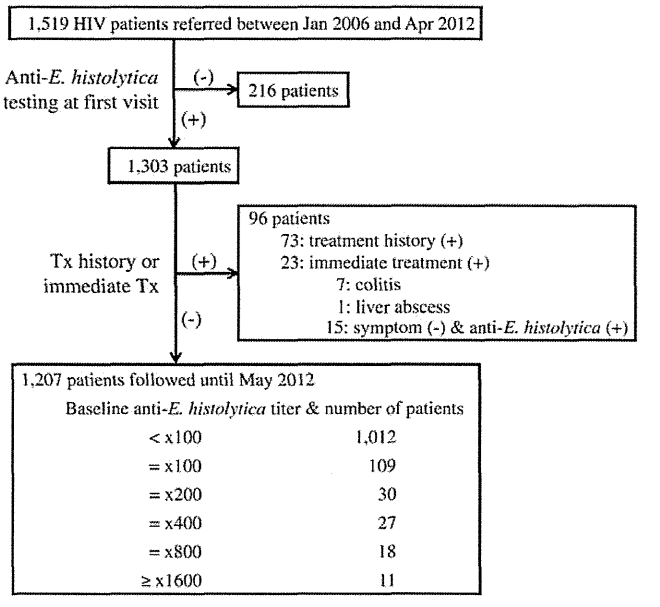

Figure 1. Flow diagram of patient recruitment process. Abbreviations: Anti-*E. histolytica*, anti-*Entamoeba histolytica* antibody; IA, invasive amebiasis; Tx, treatment.

Table 2. Comparison of Clinical Characteristics of Patients With and Without Invasive Amebiasis

	Amebic Colitis (n = 11)	Extraintestinal IA[a] (n = 7)	Non-IA (n = 1189)	*P* Value IA vs Non-IA
Age (years), average (SD)	35.9 (12.3)	38.2 (11.0)	37.5 (10.8)	.81
Japanese nationality, no. (%)	10 (90.9)	6 (85.7)	1068 (89.8)	.71
Male sex, no. (%)	11 (100)	7 (100)	1119 (94.1)	.62
MSM, no. (%)	11 (100)	6 (85.7)	929 (78.1)	.15
TPHA test-positive, no. (%)	5 (45.5)	2 (28.6)	451/1175 (38.4)	.91
HBV exposure,[a] no. (%)	6 (54.5)	5 (71.4)	630/1178 (53.5)	.15
HCV Ab-positive, no. (%)	0/11 (0)	0/7 (0)	42/1172 (3.6)	1.00
Anti-*E. histolytica* at baseline, median (IQR)	×100 (<×100–×800)	×400 (×100–×400)	<×100 (<×100–<×100)	<.001
Anti-*E. histolytica* at the onset of IA, median (IQR)	×800 (×200–×800)	×400 (×100–×800)	. . .	
Follow-up period, median months (IQR)	7.8 (3.3–25.1)	10.5 (4.9–17.9)	25.5 (7.0–47.3)	

Data were compared using χ^2 test, Student *t* test, or Mann-Whitney *U* test for qualitative or quantitative variables, respectively.

Abbreviations: Ab, antibody; Anti-*E. histolytica*, anti *Entamoeba histolytica* antibody; HBV, hepatitis B virus; HCV, hepatitis C virus; IA, invasive amebiasis; IA, invasive amebiasis; IQR, interquartile range; MSM, men who have sex with men; SD, standard deviation; TPHA, *Treponema pallidum* hemagglutination.[a]Extraintestinal cases include one case of appendicitis and 6 cases of liver abscess.

(confirmed by identification of erythrophagocytic trophozoites in surgically removed specimen), amebic liver abscess in 6, and amebic colitis in 11 (confirmed by identification of erythrophagocytic trophozoites in stool samples). The median anti-*E. histolytica* titer at baseline was significantly higher among patients who developed invasive amebiasis than that among those who did not, but the other clinical and laboratory parameters were not different between the 2 groups (Table 2). Although no significant differences in the frequency of invasive amebiasis were evident in patients with ×100 ($P = .77$) and ×200 ($P = .18$) anti-*E. histolytica* titers at baseline, compared with negative anti-*E. histolytica* patients (<×100), the frequency was higher in patients with ×400 ($P < .001$), ×800 ($P = .025$), and ≥×1600 ($P < .001$) anti-*E. histolytica* titers at baseline, compared with negative anti-*E. histolytica* patients. Univariate and multivariate analyses also showed that future development of invasive amebiasis correlated only with high titer of anti-*E. histolytica* antibody at baseline (≥×400: Univariate, HR: 20.985, 95% confidence interval [CI], 8.085–54.467; multivariate, HR: 22.079, 95% CI, 7.964–61.215) (Table 3). Furthermore, the risk of development of invasive amebiasis was significantly higher in the high anti-*E. histolytica* titer group (patients with anti-*E. histolytica* titer ≥×400 at baseline) than in the low anti-*E. histolytica* titer group (patients with anti-*E. histolytica* titer ≤×200 at baseline; log-rank test: $\chi^2 = 80.203$, $P < .001$, Kaplan-Meier estimate, Figure 2). Moreover, most patients of the high anti-*E. histolytica*

Table 3. Risk Analysis for Development of Invasive Amebiasis by Cox Proportional Hazard Regression Model

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
older age (by 1 y)	0.989 (.947–1.033)	.624		
Japanese nationality	1.334 (.305–5.840)	.702		
Male sex	21.884 (.002–241297.39)	.516		
MSM	4.318 (.573–32.518)	.156	4.048 (.488–33.584)	.195
TPHA test-positive	0.901 (.348–2.335)	.831		
HBV exposure-positive	2.183 (.778–6.124)	.138	1.839 (.644–5.249)	.255
HCV Ab-positive	0.047 (.000–2697.344)	.584		
Anti-*E. histolytica* titer ≥×400	20.985 (8.085–54.467)	<.001	22.079 (7.964–61.215)	<.001

The Cox proportional-hazards regression analysis was used to estimate the impact of anti-*E. histolytica* titer at baseline on the incidence of invasive amebiasis. The impact of basic clinical characteristics, such as sexuality and serology status of other STIs, was estimated with univariate Cox proportional hazards regression. Multivariate Cox hazards regression analysis using variables identified in univariate analysis with *P* values of < .20. In all analyses, statistical significance was defined as *P* value of < .05.

Abbreviations: Ab, antibody; Anti-*E. histolytica*, anti *Entamoeba histolytica* antibody; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; IA, invasive amebiasis; IA, invasive amebiasis; IQR, interquartile range; MSM, men who have sex with men; TPHA, *Treponema pallidum* hemagglutination.

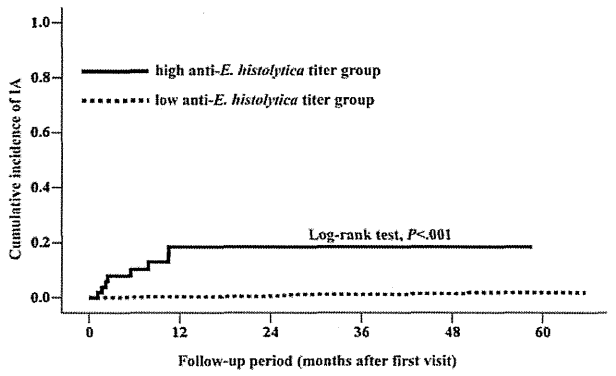

Figure 2. Incidence of invasive amebiasis in low and high anti-*E. histolytica* titer groups. Differences in the time from first visit to the diagnosis of invasive amebiasis (IA) between the low anti-*E. histolytica* titer group (≤×200 at baseline) and high anti-*E. histolytica* titer group (≥×400 at baseline) were analyzed by Kaplan-Meier method. Log-rank test was used to determine the statistical significance. Abbreviations: Anti-*E. histolytica*, anti-*Entamoeba histolytica* antibody; IA, invasive amebiasis.

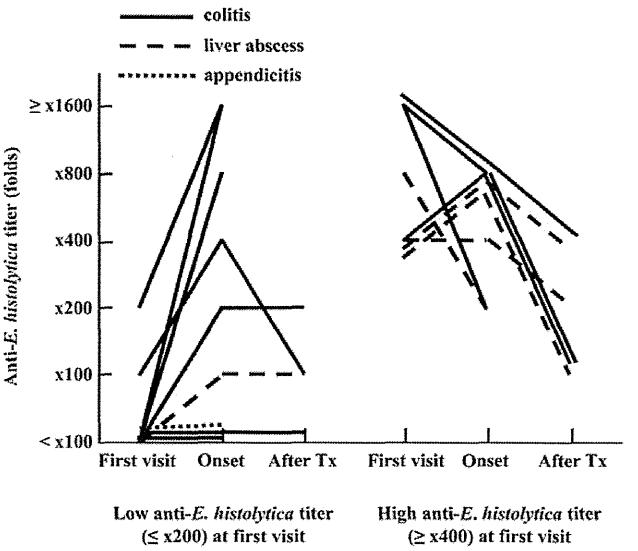

Figure 3. Anti-*E. histolytica* titer before and after diagnosis of invasive amebiasis. Anti-*E. histolytica* titer at the onset of IA was compared to that at baseline (first visit to the clinic) by Wilcoxon signed-rank test. Anti-*E. histolytica* titers after treatment were measured at 219 days [range: 174–252] and 367 days [272–841] after the completion of treatment of patients with low and high anti-*E. histolytica* titer at first visit, respectively. Abbreviations: Anti-*E. histolytica*, anti-*Entamoeba histolytica* antibody; IA, invasive amebiasis.

titer group developed invasive amebiasis during the first year of follow-up, whereas those of the low anti-E. histolytica titer group developed this complication more lately and new cases of invasive amebiasis were diagnosed throughout the follow-up period.

Transitional Changes in Anti-*E. histolytica* Titer Among Patients Who Developed Amebiasis

The median anti-*E. histolytica* titer was significantly higher at the onset of invasive amebiasis than that at first visit in patients with low baseline anti-*E. histolytica* titer (≤×200; $P = .028$, Wilcoxon signed-rank test) (Figure 3). In contrast, the median anti-*E. histolytica* titers at these 2 time points were not different in patients with high baseline anti-*E. histolytica* titer (≥×400; $P = .18$, Wilcoxon signed-rank test). Serum samples taken after nitroimidazole treatment (median time from the commencement of treatment 289 days [range 174–841]) were available in 10 patients. Anti-*E. histolytica* titers were lower after the treatment in 7 of the 10 patients, compared with the baseline values. To define the natural decay of anti-*E. histolytica*, we measured serum anti-*E. histolytica* titers at 9 months after study enrollment in 37 patients with high anti-*E. histolytica* titer at baseline but did not develop invasive amebiasis during the study period. The titers were lower, or similar to the baseline in 19 and 15 patients, respectively, whereas the remaining 3 patients showed 2-fold increase in the titer.

DISCUSSION

In the present study, the seroprevalence of anti-*E. histolytica* antibody among HIV-1-infected patients was 21.3%, which was much higher than those reported in other developed countries where amebiasis is considered as an STI [3, 9, 23, 24]. In addition, our results showed that sexually active MSM tend to be seropositive for *E. histolytica* infection, in agreement with previous studies from our group [27, 28].

The pathogenesis of amebiasis, such as incubation period after cyst ingestion and the mechanism of spontaneous remission, remains unclear. Although previous study showed anti-*E. histolytica*-positive children were more susceptible to *E. histolytica* infection than their seronegative counterparts [31], the clinical significance of anti-*E. histolytica* seropositivity and its titer in asymptomatic individuals had not been fully assessed. We measured serum anti-*E. histolytica* immunoglobulin M (IgM) levels in 18 patients at the onset of invasive amebiasis [32], but the level was detectable only in 3 patients with amebic colitis and 1 patient with liver abscess. The present study demonstrated that patients with high anti-*E. histolytica* titer (≥×400) at first visit developed invasive amebiasis much more frequently than those with low anti-*E. histolytica* titer (≤×200). The cumulative risk for invasive amebiasis among patients with high anti-*E. histolytica* titer at baseline rapidly increased during the first one year of follow-up but plateaued thereafter, suggesting that exacerbation of subclinical amebiasis occurs frequently within one year in these patients. On the other hand, the cumulative risk for invasive amebiasis among patients with low anti-*E. histolytica* titer at baseline increased more slowly and