IL-6 levels have been assayed using enzyme-linked immunosorbent assays (ELISAs), whereas Haddow *et al.* [4] used a multiplex bead array assay (MBAA). Plasma IL-6 levels in HIV patients may differ significantly when assayed by ELISA or MBAA [9].

It is now clear that assays of selected biomarkers can assist in both the prediction and diagnosis of TB-IRIS and ART-TB. Large-scale studies are now needed to delineate the value of these assays as diagnostic tests. In doing this, consideration should be given to the use of both plasma and IGRA plasma from antigen-stimulated and antigen-unstimulated tubes and also to the method used to assay cytokines and chemokines.

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Conflicts of interest

There are no conflicts of interest.

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Antiretroviral therapy alone resulted in successful resolution of large idiopathic esophageal ulcers in a patient with acute retroviral syndrome

Esophageal ulceration is a well known complication of end-stage HIV infection, and also patients with acute retroviral syndrome [1–5]. Various infectious agents can cause esophageal diseases, including *Candida* species, cytomegalovirus (CMV), and herpes simplex virus (HSV). However, in a large proportion of patients with esophageal ulcers, no agent can be identified and ulceration is considered idiopathic [1,2]. Here, we report a case of an idiopathic esophageal ulcer with concomitant primary HIV-1 infection.

A previously healthy 36-year-old Japanese male homosexual presented with odynophagia, fever, and headache. Five days after the onset, he was diagnosed with oral candidiasis and treated with fluconazole for 5 days. Despite the treatment, odynophagia did not improve. The result of voluntary HIV screening 1 month before the onset of the symptoms was negative. However, 8 days after the initial presentation, ELISA for anti-HIV antibody was positive. The patient was referred to our hospital 2 weeks after the onset of odynophagia.

On admission, the patient was alert and oriented with body temperature of 38.5°C. Physical examination showed no oral candidiasis, peripheral lymphadenopathy, or rashes. The patient could not swallow any solid food due to the severe odynophagia. Laboratory tests at admission showed elevated liver enzymes (aspartate aminotransferase, 335 IU/l; alanine aminotransferase, 357 IU/l) and elevated lactate dehydrogenase (1182 IU/l). Cerebrospinal fluid analysis showed almost normal findings [leukocyte count, 5.9 cells/μl (61% mononuclear cells); glucose level, 52 mg/dl (serum glucose, 100 mg/dl); and protein, 29 mg/dl]. Gram and India ink staining were negative, as were bacterial and mycobacterial cultures and the cryptococcal antigen test. Serum immunoglobulin

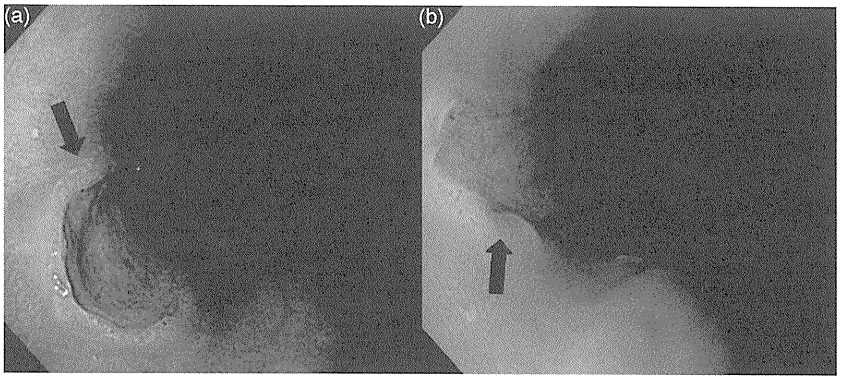

Fig. 1. Endoscopic appearance of idiopathic esophageal ulcer. Before antiretroviral therapy (a) and on the fifth day of antiretroviral therapy (b). The black arrow indicates the esophageal ulcer.

(IgG) and IgM antibodies for CMV were positive, and IgG was positive and IgM was negative for HSV. Whole blood PCR was negative for both CMV and HSV.

Western blotting for HIV antibodies revealed positivity only for GP41. Plasma HIV-1 RNA viral load was 2 200 000 copies/ml. The $CD4^+$ and $CD8^+$ cell counts were 140 and 1057 cells/μl, respectively.

Endoscopy on day 3 identified five large, discrete, and well circumscribed esophageal ulcers, with the largest measuring 15 mm in diameter Fig. 1a. There was no evidence of candidiasis. Three biopsies were obtained from the ulcers and histopathology revealed nonspecific findings: infiltration by numerous neutrophils into the squamous epithelium and lamina propria. No intranuclear or intracytoplasmic inclusion bodies were found. Immunohistochemical staining for CMV and HSV was negative.

Antiretroviral therapy of lopinavir/ritonavir with tenofovir/emtricitabine was initiated on the fourth day of admission. The following day, the patient became afebrile, and 2 days later the odynophagia and headache disappeared. Endoscopy on day 5 of antiretroviral therapy demonstrated substantial reduction in the size and depth of esophageal ulcers (Fig. 1b). The patient was discharged on day 13. One month after discharge, all bands of western blot assay turned positive, confirming the diagnosis of acute retroviral syndrome. Plasma HIV-1 RNA became undetectable 3 months after initiation of antiretroviral therapy.

To our knowledge, this is the first report demonstrating the resolution of idiopathic esophageal ulcers on antiretroviral therapy alone in a patient with untreated HIV infection. Steroids are the standard treatment of idiopathic esophageal ulcers [2,6]. However, due to their immunosuppressive effects and various other adverse effects, they are not generally recommended in immunocompromised patients. The cause of idiopathic esophageal ulcer is considered to include HIV-associated T-cell activation, which induces apoptosis of esophageal mucosa [5,7]. This probable cause supports the rationale of using antiretroviral therapy for idiopathic esophageal ulcer.

The diagnosis of idiopathic esophageal ulcer is accomplished by excluding other infectious agents known to cause esophageal ulceration, notably CMV and HSV, by histopathological and immunological examinations of endoscopically obtained specimens [2,8]. Pill-induced esophagitis and gastroesophageal reflux disease need to be excluded [2]. The presented case was positive for anti-CMV IgM antibody, suggestive of primary infection or reactivation of CMV. It is, thus, difficult to completely exclude CMV-related esophageal ulceration in this patient. However, histopathology showed no evidence of CMV-related cytopathic changes, and immunohistochemical staining for CMV was also negative. Furthermore, initiation of antiretroviral therapy resulted in rapid healing of the esophageal ulcers, suggesting that primary HIV infection was the cause of ulceration. It is noteworthy that anti-CMV IgM antibody could become falsely positive during acute infection with other viruses presumably because of shared epitopes among other antigens [9,10]. Thus, idiopathic esophageal ulcer is the most probable diagnosis in this case.

It is concluded that in untreated patients with HIV lacking identifiable infectious agents, such as CMV or HSV, in endoscopic biopsies from esophageal ulcer, antiretroviral therapy alone could produce a favorable outcome.

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ORIGINAL

Prevalence of and risk factors for lipodystrophy among HIV-infected patients receiving combined antiretroviral treatment in the Asia-Pacific region: results from the TREAT Asia HIV Observational Database (TAHOD)

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Abstract. The prevalence of and risk factors for lipodystrophy (LD) among patients receiving combined antiretroviral treatment (cART) in the Asia-Pacific region are largely unknown. LD diagnosis was based on the adverse event definition from the US NIH Division of AIDS (2004 version), and only cases with a severity grade of ≥ 3 were included. TAHOD patients who had recently commenced cART with ≥ 3 drugs after 1996 from sites which had ever reported LD were included in the analysis. Covariates for the forward multivariate logistic regression model included demographic variables, CDC disease classification, baseline CD4 and viral load, hepatitis B/C virus co-infection, and regimen and duration of cART. LD was diagnosed in 217 (10.5%) of 2072 patients. The median duration of cART was 3.8 (interquartile range, 2.2-5.3) years (stavudine, 2.0 (1.0-3.5) years; zidovudine, 1.8 (0.6-3.9) years; and protease inhibitors (PI), 2.6 (1.3-4.5) years). In the multivariate model, factors independently associated with LD included use of stavudine (≤ 2 years *vs.* no experience: OR 25.46, $p<0.001$, > 2 years *vs.* no experience: OR 14.92, $p<0.001$), use of PI (> 2.6 years *vs.* no experience: OR 0.26, $p<0.001$), and total duration of cART (> *vs.* ≤ 3.8 years: OR 4.84, $p<0.001$). The use of stavudine was strongly associated with LD in our cohort. Stavudine-sparing cART strategies are warranted to prevent the occurrence of LD in the Asia-Pacific region.

Key words: Lipodystrophy, HIV, Adverse effects, Combined antiretroviral treatment, Asia-Pacific

SINCE morphologic changes caused by fat redistribution was first reported in subjects receiving combined antiretroviral treatment (cART) in 1998 [1], lipodystrophy (LD) has become a recognized

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Statistical Abbreviations used: IQR, interquartile range; OR, odds ratio; CI, confidence interval.

complication in HIV-infected patients on cART around the world, with a prevalence ranging from 11% to 83% [2]. LD is acknowledged as an important adverse event in HIV-infected subjects in the era of cART, because it is associated with other metabolic abnormalities such as insulin resistance, dyslipidemia, and glucose intolerance, attributing to the development of cardiovascular disease [2]. In severe cases, LD can be disfiguring, which can cause stigma and discrimination against patients, leading to risks of medication refusal, poor adherence, and ultimately treatment failure [2-5].

Several characteristics, including older age, white race, lower body mass index (BMI), higher HIV-RNA levels, lower nadir CD4+ T-cell count, longer duration of cART and exposure of nucleoside reverse transcriptase inhibitors (NRTIs), especially stavudine (d4T), have been identified as risk factors for LD [2, 6]. Also, recent studies have reported that genetic variations can also influence the emergence of LD [7-9].

In East, South, and South-East Asia, the estimated number of people living with HIV and/or acquired immunodeficiency syndrome (AIDS) in 2007 was 5 million, the second largest regional epidemic in the world, after Sub-Saharan Africa [10]. Except for a small number of single-institution studies, there is little data on the prevalence and risk factors of LD among HIV-infected patients receiving cART in the Asia-Pacific region [11-14].

The Therapeutics Research, Education, and AIDS Training in Asia (TREAT Asia) HIV Observational Database (TAHOD) is a multicenter, observational cohort study that was initiated in 2003 to assess regional HIV treatment outcomes in the Asia-Pacific region [15]. Our objective was to examine the prevalence of and the risk factors for LD among HIV-infected patients receiving cART in TAHOD.

Materials and Methods

Study design and patient population

The structure of TAHOD and standardized mechanisms for data collection and follow-up has been previously described [15]. Data were combined *via* standardized formats and transferred electronically to the National Centre in HIV Epidemiology and Clinical Research (NCHECR), University of New South Wales, Sydney, Australia for central aggregation, quality control, and analyses. Ethics approval was obtained from the University of New South Wales and the local ethics committee for each site. Because all data transferred to the NCHECR were collected in an anonymous fashion and entirely observational, informed consent was not obtained, unless specifically requested by the local ethics committee.

Observational TAHOD data aggregated to NCHECR by April 2008, involving 17 institutions in 12 countries (Appendix), were included in this study. Patients who had recently commenced cART with ≥ 3 antiretroviral drugs after 1996 in any TAHOD participating sites which had ever reported LD were eligible for the analysis. Patients who had started treatment with < 3 antiretroviral drugs before 1996 were not included in this study.

Because TAHOD is a multicenter observational database, not all patients included in this analysis were receiving cART according to standardized guidelines. The timing of antiretroviral treatment and the regimens of combined antiretroviral drugs were decided upon by individual physicians depending on unique clinical circumstances.

Data collection and definitions

The following were included as covariates: age, gender, race, country income category, reported mode of transmission, hepatitis B and C virus (HBV/HCV) co-infection status, and baseline and monitoring values at and after start of cART (e.g., age, US Centers for Disease Control and Prevention (CDC) disease classification [16], CD4+ T-cell and HIV-RNA viral load, BMI, and cART regimen and duration). Country income category was divided into two groups based on the 2007 gross national income per capita, according to the World Bank criteria for classifying economies: low income country ($3,705 or less), and high income country ($3,706 or more) [17].

In TAHOD, LD data was collected as 1) fat accumulation according to a clinical spectrum of central fat accumulation in the abdomen, breasts, and over the dorsocervical spine, or localized lipomas and/or 2) lipoatrophy according to a clinical spectrum of peripheral fat loss in the face, limbs, or buttocks. LD was diagnosed based on the clinical definition of the US Division of AIDS table for grading the severity of adult and pediatric adverse events (2004 version) [18]. Patients with a severity of grade 3, defined as disfiguring or obvious body shape changes on casual visual inspection, or higher were included in this analysis [18].

Statistical analysis

Continuous data were represented using the median value (IQR) and categorical variables were reported by number (percent). cART-related covariates were analysed as 1) never treated, 2) below the median duration (MD) of treatment, and 3) above the MD of treatment. Associations of treatment duration between other antiretroviral drugs and d4T were evaluated using Spearman's correlation coefficient ρ. The difference in the number of patients who had an exposure history to both zidovudine (AZT) and d4T among the three groups according to time on AZT treatment was evaluated using a one-way ANOVA test.

Predictors associated with diagnosis of LD were assessed by forward, stepwise multivariate conditional logistic regression models. To control for different clinical practices in LD diagnosis, the final model was stratified by TAHOD sites. All variables with p-value of less than 0.10 in univariate analyses were included in the multivariate logistic models. All statistical analyses were performed using the STATA package (version 8.2, StataCorp, College Station, TX, USA). All p-values were two-tailed, and $p<0.05$ was considered to be statistically significant.

Results

Demographic and clinical characteristics of all study participants and in patients with LD

12 of the 17 participating TAHOD sites had previously reported LD according to our criteria and were included in the analysis. LD was diagnosed in 217 (10.5%) patients among a total of 2,072 study participants. Upon univariate analysis, the prevalence of LD did not differ significantly according to age, gender, race, HBV co-infection status, CD4+ T-cell count or HIV-RNA levels, or BMI at cART initiation. Patients with HCV co-infection had a significantly lower prevalence of LD than those without (5% *vs.* 12%; OR, 0.37; p=0.019). The rate of LD in patients living in high-income countries was lower than that of patients living in low-income countries, though the difference was not statistically significant (5% *vs.* 13%; OR, 0.32; p=0.095) (Table 1).

Table 1 Demographic and clinical characteristics of all study participants and patients with lipodystrophy

	Total (n=2072)	Lipodystrophy (n=217)	Univariate OR	Univariate *p*-value
Age (years) at cART initiation				
Median (IQR)	35 (30-41)	36 (31-41)		
≤ 30*	549 (27)	45 (8)	–	–
31~40	954 (46)	110 (12)	1.32	0.170
≥ 41	569 (27)	62 (11)	1.41	0.129
Gender				
Male*	1485 (72)	127 (9)	–	–
Female	587 (28)	90 (15)	1.20	0.330
Race				
Chinese	871 (42)	50 (6)	–	–
Thai	434 (21)	128 (29)	1.74	0.481
Indian	207 (10)	31 (15)	1.02	0.980
Other	560 (27)	8 (1)	0.89	0.849
Hepatitis B surface antigen				
Negative*	1257 (61)	144 (11)	–	–
Positive	164 (8)	15 (9)	0.76	0.378
Not tested	651 (31)	58 (9)	0.60	0.025
Hepatitis C antibody				
Negative*	1236 (60)	145 (12)	–	–
Positive	138 (7)	7 (5)	0.37	0.019
Not tested	698 (33)	65 (9)	0.68	0.066
CDC classification at cART initiation				
Category A*	1000 (48)	93 (9)	–	–
Category B	200 (10)	23 (12)	1.94	0.030
Category C	872 (42)	101 (12)	1.07	0.669
CD4 count (cells/μL) at cART initiation				
Median (IQR)	103 (33-198)	70 (25-152)		
≤ 100*	764 (37)	96 (13)	–	–
101~200	414 (20)	36 (9)	0.96	0.842
201~300	235 (11)	23 (10)	1.50	0.141
≥ 301	135 (7)	7 (5)	1.14	0.760
Not tested	524 (25)	55 (11)	1.74	0.025
HIV viral load (copies/mL) at cART initiation				
Median (IQR) – Log_{10}	4.9 (4.3-5.5)	4.9 (4.3-5.5)		
≥ 400*	690 (33)	56 (8)	–	–
< 400	56 (3)	3 (5)	0.79	0.713
Not tested	1326 (64)	158 (12)	0.97	0.891
BMI (kg/m^2) at cART initiation				
Median (IQR)	20.3 (18.3-22.7)	20.4 (18.6-22.5)		
≤ 18.5*	202 (10)	23 (11)	–	–
18.5~25	437 (21)	63 (14)	1.44	0.218
> 25	73 (4)	9 (12)	1.70	0.277
Not available	1360 (65)	122 (9)	1.79	0.097
Country income category				
Low-income*	1452 (70)	189 (13)	–	–
High-income	620 (30)	28 (5)	0.32	0.095

Data are expressed as median (interquartile range) or number (percent). *Reference category. Abbreviations used: OR, odds ratio; cART, combined antiretroviral treatment; IQR, interquartile range; BMI, body mass index.

LD prevalence by cART exposure

The majority (72%) of patients received non-nucleoside reverse transcriptase inhibitors (NNRTI)-based cART as their first-line regimen; among these,

41% received efavirenz (EFV) and 59% nevirapine. There was no difference in the prevalence of LD between patients who used NNRTI or protease inhibitor (PI) as their first antiretroviral regimen (12% *vs.* 8%; OR, 0.66; p=0.499). The median duration of cART in all the patients was 3.8 years overall, 1.8 years on AZT, 2.0 years on d4T, 1.9 years on didanosine (ddI), 2.9 years on any NNRTI, and 2.6 years on any PI. The patients receiving cART for > 3.8 years had a significantly higher prevalence of LD than those receiving cART for ≤ 3.8 years (16% *vs.* 5%; OR, 4.01; p=0.001). In addition, patients who had received d4T had a significantly higher prevalence of LD than those who had never received d4T (> 2 years *vs.* no experience [NE], 19% *vs.* 1%; OR, 41.01; p<0.001, and ≤ 2 years *vs.* NE, 12% *vs.* 1%; OR, 23.55; p<0.001]. However, patients who received AZT for > 1.8 years had a significantly lower prevalence of LD than those who had never received AZT (3% *vs.* 8%; OR, 0.34; p=0.003). Patients who received any PI for > 2.6 years had a significantly lower prevalence of LD than those who had never received any PI (3% *vs.* 13%; OR, 0.20; p=0.010) (Table 2).

In the group receiving d4T for > 2 years, patients with NNRTI-based cART had a higher, but not statistically significant, rate of LD than those with PI-based cART (20.7% *vs.* 14.4%; p=0.098). There were no significant differences in the rates of LD between NNRTI- and PI-based cART by d4T exposure (Fig. 1). The duration of AZT or PI use had a significantly negative correlation with duration of d4T treatment (r= -0.43; p<0.001, r= -0.06; p=0.006, respectively) (Table 3). The group of those who had received AZT for ≤ 1.8 years (514 of 690, 74.5%) had a significantly larger proportion of patients who had a history of both AZT and d4T use than did the groups who had never received AZT (0 of 692, 0%, p<0.001) or who had received AZT for > 1.8 years (285 of 690, 41.3%, p<0.001).

Independent predictive factors associated with the diagnosis of LD

In the multivariate logistic regression model, factors independently associated with LD included the use of d4T (≤ 2.0 years *vs.* NE, OR 25.46, 95% CI 9.01-71.97, p<0.001; and > 2.0 years *vs.* NE, OR 14.92, 95% CI 5.29-42.06, p<0.001), use of PI (> 2.6 years *vs.* NE, OR 0.26, 95% CI 0.12-0.56, p<0.001), and the total duration of cART (> *vs.* ≤ 3.8 years, OR 4.84, 95% CI 3.11-7.55, p<0.001) (Table 4).

Discussion

In general, the prevalence rates of LD were reported with ranges from 7 to 84% (average of 42%) in patients receiving PI-based cART, and from 0 to 38% (average of 13%) in those receiving NNRTI-based cART from various populations and countries with different clinical and metabolic characteristics [19]. This study, based on data from a multicenter observational database in the Asia-Pacific region, showed a lower prevalence of LD than the rates of 43 to 53% seen earlier in the cART era in large cohorts in Europe, Australia, and the United States [20-22]. However, in a Spanish cohort reported more recently, the prevalence of LD was 17.8% (420 of 2358) [23] and, according to data from a Swiss HIV study, patients starting cART in 2003-2006 were significantly less likely to experience LD than those starting between 2000 to 2002 [24]. As treatment patterns change with a decrease in thymidine analogue (d4T/AZT/ddI) use and an increase in tenofovir disoproxil fumarate (TDF) use, it is likely that LD rates will also decline [24].

To our knowledge, our analysis is the first regional cohort study of LD in the Asia-Pacific. Previous single-institution studies in Thailand, Singapore, and South Korea reported the prevalence of LD as ranging from 3.5% to 66.1% [11-14]. Because LD was identified clinically using a high threshold (i.e., severity grade ≥ 3) and without the use of quantitative tools such as dual-energy X-ray absorptiometry (DEXA) or computerized tomography (CT) scans, the true prevalence of LD may be higher than what we have reported. Also, the lower prevalence of LD in our sites might have been caused by different host factors such as unknown genetic background and insufficient concern for LD in several resource-limited settings. Actually, a few previous studies showed that LD was infrequent in non-white races as compared to caucasians [22, 25, 26], and that genetic variations can also influence the emergence of LD [7-9]. Further studies are warranted to confirm a more objective prevalence of LD through universalized and validated case definitions in the Asia-Pacific region.

We confirmed that the use of d4T is a strong risk factor for the development of LD in the Asia-Pacific region. Our results are consistent with other studies that show that treatment with NRTIs, and particularly with d4T, which has the greatest mitochondrial toxicity in the class, and longer duration of cART are key risk factors for the development of LD [20, 24, 26, 27]. The

Table 2. Prevalence of lipodystrophy according to combined antiretroviral treatment

	Total (n=2072)	Lipodystrophy (n=217)	Univariate	
			OR	*p*-value
Total duration on cART (years)	3.8 (2.2-5.3)	4.9 (4.0-5.9)		
≤ 3.8 years*	1036 (50)	48 (5)	-	-
> 3.8 years	1036 (50)	169 (16)	4.01	0.001
First-line combination				
NNRTI-based cART*	1497 (72)	175 (12)	-	-
PI-based cART	522 (25)	42 (8)	0.66	0.499
Other	53 (3)	0 (0)	-	-
Time on NRTI treatment				
Time on zidovudine (years)	1.8 (0.6-3.9)	0 (0-0.2)		
Never received*	692 (34)	53 (8)	-	-
≤ 1.8 years	690 (33)	145 (21)	3.21	0.168
> 1.8 years	690 (33)	19 (3)	0.34	0.003
Time on stavudine (years)	2.0 (1.0-3.5)	2.4 (1.6-3.3)		
Never received*	699 (34)	4 (1)	-	-
≤ 2.0 years	687 (33)	82 (12)	23.55	<0.001
> 2.0 years	686 (33)	131 (19)	41.01	<0.001
Time on didanosine (years)	1.9 (0.9-3.4)	1.8 (1.0-2.6)		
Never received*	1519 (74)	166 (11)	-	-
≤ 1.9 years	277 (13)	29 (10)	0.95	0.938
> 1.9 years	276 (13)	22 (8)	0.71	0.544
Time on NNRTI treatment (years)	2.9 (1.7-4.4)	2.7 (1.7-3.6)		
Never received*	355 (17)	25 (7)	-	-
≤ 2.9 years	859 (42)	115 (13)	2.04	0.399
> 2.9 years	858 (41)	77 (9)	1.30	0.759
Time on PI treatment (years)	2.6 (1.3-4.5)	1.6 (0.7-2.5)		
Never received*	1347 (65)	169 (13)	-	-
≤ 2.6 years	363 (18)	38 (10)	0.82	0.764
> 2.6 years	362 (17)	10 (3)	0.20	0.010
Time on atanazavir (years)	1.4 (0.7-1.6)	0 (0-0)		
Never received*	1879 (90)	216 (12)	-	-
≤ 1.4 years	97 (5)	1 (1)	0.08	0.005
> 1.4 years	96 (5)	0 (0)	-	-
Time on indinavir (years)	2.0 (0.9-3.6)	1.6 (0.6-2.3)		
Never received*	1714 (82)	175 (10)	-	-
≤ 2.0 years	179 (9)	30 (17)	1.77	0.366
> 2.0 years	179 (9)	12 (7)	0.37	0.434
Time on nelfinavir (years)	2.1 (0.7-4.0)	0.6 (0.1-1.1)		
Never received*	1951 (94)	205 (11)	-	-
≤ 2.1 years	61 (3)	12 (20)	2.09	0.216
> 2.1 years	60 (3)	0 (0)	-	-
Time on lopinavir (years)	1.8 (0.6-3.3)	1.2 (1.0-1.3)		
Never received*	1798 (86)	213 (12)	-	-
≤ 1.8 years	138 (7)	4 (3)	0.22	0.007
> 1.8 years	136 (7)	0 (0)	-	-

Data are expressed as median (interquartile range) or number (percent). NNRTI-based cART indicates antiretroviral treatment composed of a combination of more than two NRTI and one NNRTI antiretroviral drugs, but without PI; and PI-based cART indicates the antiretroviral treatment which is composed of the combination of more than two NRTI and PI antiretroviral drugs, but without NNRTI. *Reference category. Abbreviations used: OR, odds ratio; cART, combined antiretroviral treatment; IQR, interquartile range; NRTI, nucleoside analogue reverse transcriptase inhibitor; NNRTI, non-nucleoside analogue reverse transcriptase inhibitor; PI, protease inhibitor.

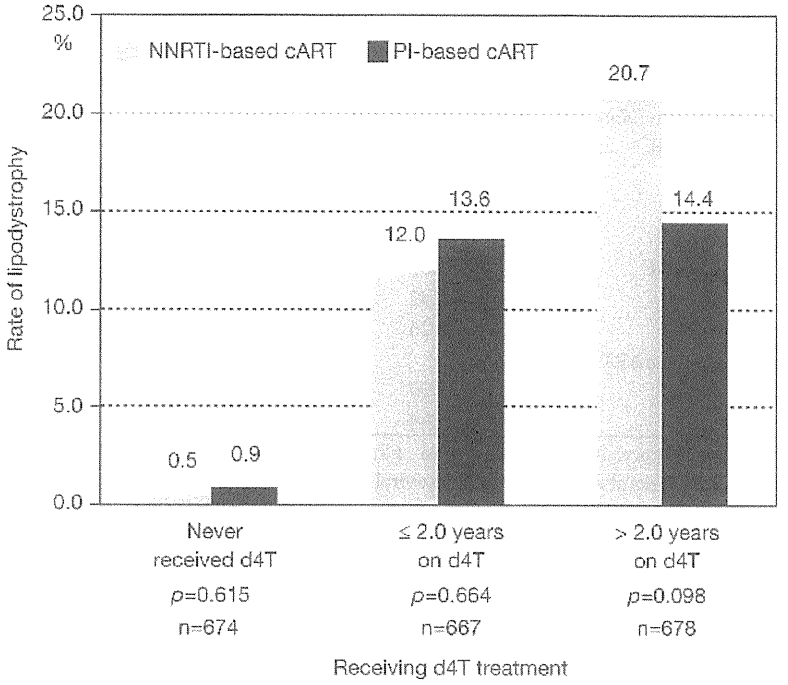

Fig. 1 The prevalence of lipodystrophy between NNRTI-based and PI-based combined antiretroviral treatment in each group, according to time on stavudine treatment
NNRTI-based cART indicates antiretroviral treatment composed of a combination of more than two NRTI and one NNRTI antiretroviral drugs, but without PI; and PI-based cART indicates the antiretroviral treatment which is composed of the combination of more than two NRTI and PI antiretroviral drugs, but without NNRTI. Abbreviations used: NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; cART, combined active antiretroviral treatment; d4T, stavudine; NRTI, nucleoside reverse transcriptase inhibitor.

Table 3 Association with other antiretroviral drugs treatment duration and exposure duration of stavudine in total patients (n=2072)

	Exposure duration of stavudine (months)		
	Median (IQR)	Spearman's ρ	*p*-value
Time on zidovudine treatment		-0.43	<0.001
Never received	23.6 (5.9-44.0)		
≤ 1.8 years	14.6 (0-34.3)		
> 1.8 years	0 (0-13.7)		
Time on didanosine treatment		0.14	<0.001
Never received	10.0 (0-31.3)		
≤ 1.9 years	13.7 (4.1-30.1)		
> 1.9 years	25.4 (0-44.9)		
Time on NNRTI treatment		0.31	<0.001
Never received	0 (0-18.6)		
≤ 2.9 years	9.4 (0-22.8)		
> 2.9 years	28.8 (0-47.4)		
Time on PI treatment		-0.06	0.006
Never received	13.1 (0-33.9)		
≤ 2.6 years	12.2 (0-24.3)		
> 2.6 years	10.4 (0-35.1)		

Abbreviations used: NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Table 4 Forward multivariate logistic regression analysis to identify the risk factors for lipodystrophy

Covariate	Odds ratio	95% CI	*p*-value
Experience on stavudine			
Never received*	1.00	-	-
≤ 2.0 years	25.46	9.01-71.97	<0.001
> 2.0 years	14.92	5.29-42.06	<0.001
Experience on PI			
Never received*	1.00	-	-
≤ 2.6 years	1.42	0.86-2.34	0.165
> 2.6 years	0.26	0.12-0.56	<0.001
Total duration on cART			
≤ 3.8 years*	1.00	-	-
> 3.8 years	4.84	3.11-7.55	<0.001

*Reference category. Abbreviations used: CI, confidence interval; PI, protease inhibitor; cART, combined active antiretroviral treatment.

finding that patients receiving AZT for longer than the MD of treatment were less likely to have LD may have been due to their relatively shorter exposure to d4T.

Our finding that patients living in high-income countries had a tendency toward a lower prevalence of LD than those in low-income countries might have been related to the wider range of available antiretroviral drugs and lower reliance on d4T in high-income countries.

It was unexpected that patients with longer PI treatment would have lower risk of developing LD, regardless of the duration of d4T treatment. Treatment with PIs is known to be an important attributable factor for the development of LD, and NNRTIs were not traditionally considered to be associated with the development of LD [24, 28, 29]. However, recent clinical trials have revealed that limb fat gain in patients receiving EFV was lower than in treatment with ritonavir-boosted lopinavir (LPV/r) [29-32]. In addition, a recent AIDS Clinical Trials Group (ACTG) A5142 study showed that lipoatrophy was more frequent with EFV than with LPV/r when combined with d4T or AZT [27, 30]. These findings suggest a protective role of ritonavir-boosted PIs for LD. The mechanisms for the protective role of ritonavir-boosted PIs are not well understood, but a potential explanation is that ritonavir may mitigate the mitochondrial damage caused by thymidine analogues [27]. Although PIs, especially ritonavir-boosted, may have potential protective roles for LD, other metabolic complications, such as dyslipidemia and insulin resistance, and the tolerances of drugs associated with PIs should be prudently considered when choosing an antiretroviral drug [27].

Our study had several limitations. The lack of a uniform, objective, diagnostic method for identifying LD could have led to a selection bias. In this study, we tried to capture the rate of LD in the Asia-Pacific sites that are capable of diagnosing LD. However, the sites themselves that have ever reported LD may add confounding factors such as antiretroviral regimens, race, and HCV infection. Although we limited the inclusion criteria to higher-grade LD to reduce this risk, cases of LD could have been missed, due to local variations in diagnosis and reporting. As this is an observational cohort across centers with varying levels of clinical and monitoring capacity, not all patients had baseline levels for all possible variables. We excluded pre-ART lipid and glucose tests from our final analysis for this reason, but acknowledge that other missing data could have impacted our findings.

Stavudine is one of the most commonly used NRTIs in the world and in the Asia-Pacific region [5, 33]. TDF is unavailable at many sites in the Asia-Pacific region, so d4T or AZT in combination with 3TC is usually the only NRTIs available for standard first-line regimens [5, 33]. Our findings emphasize the importance of phasing out d4T use and increasing access to TDF to minimize the risk of developing LD.

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Author Disclosure Statement

All authors declare that they have no conflicts of interest associated with this manuscript.

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Appendix

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PLoS NEGLECTED TROPICAL DISEASES

Amebiasis in HIV-1-Infected Japanese Men: Clinical Features and Response to Therapy

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Abstract

Invasive amebic diseases caused by *Entamoeba histolytica* are increasing among men who have sex with men and co-infection of ameba and HIV-1 is an emerging problem in developed East Asian countries. To characterize the clinical and epidemiological features of invasive amebiasis in HIV-1 patients, the medical records of 170 co-infected cases were analyzed retrospectively, and *E. histolytica* genotype was assayed in 14 cases. In this series of HIV-1-infected patients, clinical presentation of invasive amebiasis was similar to that described in the normal host. High fever, leukocytosis and high CRP were associated with extraluminal amebic diseases. Two cases died from amebic colitis (resulting in intestinal perforation in one and gastrointestinal bleeding in one), and three cases died from causes unrelated to amebiasis. Treatment with metronidazole or tinidazole was successful in the other 165 cases. Luminal treatment was provided to 83 patients following metronidazole or tinidazole treatment. However, amebiasis recurred in 6 of these, a frequency similar to that seen in patients who did not receive luminal treatment. Recurrence was more frequent in HCV-antibody positive individuals and those who acquired syphilis during the follow-up period. Various genotypes of *E. histolytica* were identified in 14 patients but there was no correlation between genotype and clinical features. The outcome of metronidazole and tinidazole treatment of uncomplicated amebiasis was excellent even in HIV-1-infected individuals. Luminal treatment following metronidazole or tinidazole treatment does not reduce recurrence of amebiasis in high risk populations probably due to amebic re-infection.

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Introduction

Invasive amebiasis (IA) caused by *Entamoeba histolytica* is the second most common cause of mortality associated with parasitic infections worldwide, accounting for 40,000 to 100,000 deaths annually [1]. Amebiasis is transmitted by ingestion of food or water containing the cyst form of *E. histolytica*, which is prevalent in developing countries in Central and South America, Asia, and Africa. In the developed countries, most cases arise in travelers and immigrants from such endemic areas [2]. Recently, however, three developed East Asian countries (Japan, Taiwan, and South Korea) reported increased risk for amebiasis among men who have sex with men (MSM) due to oral-anal sexual contact [3–12]. The annual incidence of human immunodeficiency virus type 1 (HIV-1) infection is also increasing among MSM in these countries [13-17], resulting in growing concern on IA in HIV-1-infected MSM [6,9–12,18]. The recommended treatment for IA is metronidazole (750 mg t. i. d. for 10 days) or tinidazole (2 g q. d. for 3 days), followed by a luminal agent (paromomycin 500 mg t. i. d. for 10 days or diloxanide furoate 500 mg t. i. d. for 10 days) to eliminate intestinal colonization [18,19]. A previous report described no difference in the response to metronidazole or tinidazole treatment between HIV-1-positive and –negative IA patients [20]. However, the efficacy of luminal treatment in preventing recurrence, which can arise by relapse or re-infection, has not yet been assessed rigorously. In this study, we retrospectively analyzed 170 HIV-1-infected Japanese patients with IA, together with genomic typing of *E. histolytica* in 14 of these patients, and delineated the clinical features of IA in HIV-1-infected individuals and the efficacy of metronidazole, tinidazole and luminal treatment.

Methods

Ethics statement

The Institutional Review Board of National Center for Global Health and Medicine (Tokyo, Japan) approved this study. All patients who provided clinical samples for genotyping of *E. histolytica* gave written informed consent.

Case review

The medical records of HIV-1-infected cases diagnosed with IA at the AIDS Clinical Center, National Center for Global Health and Medicine, between April 1997 and March 2010, were reviewed. The diagnosis of IA was made when one of the following criteria was satisfied; 1) identification of and/or positive PCR (methods; see below) in clinical specimens (stool or punctuate-exudate) for erythrophagocytic trophozoites in patients with IA-

Author Summary

Amebiasis is usually transmitted by ingestion of contaminated food or water in developing countries. Recently, however, increased risk for amebiasis among men who have sex with men (MSM) due to oral-anal sexual contact was reported in developed countries, resulting in growing concern on amebiasis in HIV-1-infected MSM. The recommended treatment of amebiasis is metronidazole or tinidazole, followed by a luminal agent to eliminate intestinal cyst colonization. However, the efficacy of luminal treatment in preventing recurrence has not been assessed yet. In this study, we analyzed the medical records of 170 patients with amebiasis and HIV-1 co-infection. Treatment with metronidazole or tinidazole was excellent whereas luminal treatment did not reduce the frequency of recurrence of amebiasis. Recurrence was more frequent in those MSM with signs of sexual activity such as syphilis infection. Luminal treatment following metronidazole or tinidazole treatment does not reduce recurrence of amebiasis in high risk populations.

related symptoms, e.g., fever and liver abscess, or tenesmus and diarrhea, 2) high serum titer (>1:100) for antibody against *E. histolytica* in patients with IA-related symptoms in whom microbiological cultures or histological examination of clinical specimens did not identify any pathogen, and who showed improvement of IA symptoms following metronidazole or tinidazole monotherapy [10–12]. The medical records were surveyed for patients' characteristics, presenting forms of clinical IA [e.g., colitis, amebic liver abscess (ALA), and perianal abscess], HIV-1-induced immunocompromised status, and symptoms, laboratory data and serological markers of other sexually-transmitted diseases (STD) including syphilis, hepatitis B and C viruses (HBV and HCV). After completion of treatment for IA, the medical records were followed-up until March 2010, excluding those cases found to have died or lost to follow-up.

Genotyping of *E. histolytica*

To determine the strains of *E. histolytica* among HIV-1-infected Japanese patients, genotyping of *E. histolytica* was performed in patients who were PCR positive. The PCR method was used for the first time in our clinic for the diagnosis of amebiasis in December 2008, and since then 14 patients had been diagnosed as IA based on a positive PCR. For the PCR, DNAs were extracted from various biological specimens (e.g., stool, colon wash and punctuate-exudate) by using QIAamp DNA stool Mini Kit (Qiagen, Valencia, CA). Polymerase chain reactions were performed with specific sets of primers designed to target each of 6 loci (D-A, S-Q, R-R, A-L, S^{TGA}-D, and N-K) of tRNA-linked polymorphic short tandem repeats (STR), as described previously [21]. The PCR product was sequenced by ABI 3130XL Genetic Analyzer (Applied Biosystem, Foster city, CA) in both forward and reverse directions. Phylogenetic analysis and genotyping were performed as described previously [22].

Statistical analysis

Differences in patients' characteristics and clinical features were examined using the chi-square test or nonparametric test. The cumulative risk for recurrence was analyzed by the Kaplan-Meier method, and differences were tested by the log-rank test. The Cox proportional hazards model was used to assess the impact of luminal treatment on the recurrence rate after adjustment for other factors. The hazard ratio and 95% confidence interval were calculated. *P* values less than 0.05 were considered to denote statistical significance. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL).

Results

Clinical data and response to treatment

IA was diagnosed in 170 HIV-1-infected cases between April 1997 and March 2010 (including amebic colitis, n = 102; ALA, n = 63; and perianal abscess, n = 5, Table 1). Thirty-three patients had two of the above three clinical forms of IA. All patients were males and 164/170 (96.5%) were MSM. High rates of positive TPHA (*Treponema pallidum* hemagglutination assay) (71.2%) and HBV exposure (HBs antigen-positive, HBs antibody-positive, or HBc antibody-positive) (60.0%) were observed. No significant differences were seen in CD4 counts, HIV-1 loads, coexisting AIDS definite disease and the proportion of patients treated with antiretrovirals, suggesting that HIV-induced immunocompromised status did not have an impact on the clinical presentation of amebic infection, in agreement with previous data [12]. In cases of amebic colitis (n = 102), diarrhea (69.7%) was the most common symptom followed by dysentery (55.9%) (Table 2). Fever (>37.5°C) was seen in only 20 patients (19.6%), including 5 cases with perforative peritonitis. In cases with ALA (n = 63), fever (95.2%) was the most common symptom followed by abdominal pain (55.6%). Diarrhea (46.0%) and dysentery (19.0%) were only seen in less than half of ALA cases. Single abscess (72.6%) was identified in most cases. Liver abscesses were seen more frequently in the right lobe (70.5%) than the left (9.8%). Nine patients (14.3%) had pleuritis (considered a co-existing disease), as well as abscesses in the right lobe, and 7 of these presented chest pain. Comparison of physical and laboratory data showed higher peak body temperature (BT), leukocyte count and C reactive protein (CRP) in ALA cases (Table 2) and perforative peritonitis cases (data not shown) compared with colitis cases, indicating that high fever, leukocytosis and high CRP could be the signs of extraluminal amebiasis. It is reported that high fever and leukocytosis are also common in ALA patients free of HIV-1 infection, though both parameters were unusually associated with simple amebic colitis [23]. In ALA cases, however, leukocyte count correlated positively with CD4 count (data not shown in tables: Pearson product-moment correlation coefficient 0.36, p value 0.004) and negatively with HIV-RNA load (Pearson product-moment correlation coefficient -0.28, p value 0.03), but CRP correlated neither with CD4 count nor HIV-RNA load (CRP-CD4, $p = 0.81$, CRP-HIV-RNA, $p = 0.32$). There were also no correlations between CD4 count, HIV-RNA load, BT, leukocyte count or CRP and abscess size or number.

All patients were treated with metronidazole (750 mg t. i. d. for 10 days) for IA, with the exception of two who were treated with tinidazole (2 g q. d. for 3 days). Complete remission of all IA symptoms was observed in 165 patients including the two treated with tinidazole. Five cases died within six months after diagnosis of IA; two from complications related to amebic colitis (one peritoneal perforation and one gastrointestinal bleeding), one from malignant lymphoma, one from *Pneumocystis jirovecii* pneumonia, and one from pulmonary thrombosis. The overall mortality rate was 3% in this study, which was comparable to those reported in non-HIV cases [2,23].

Recurrence after treatment

Luminal agents; paromomycin and diloxanide, are not approved in Japan, and they were not always available in our facility during the study period. After completion of IA treatment with metronidazole or tinidazole, luminal agents were administered when available. Consequently, 83 cases were treated with luminal

Table 1. Patient demographics, state of HIV, and serological markers.

	Colitis (n = 102)[1]	ALA (n = 63)[2]	Perianal abscess (n = 5)[3]	All (n = 170)	*P* value[4]
Age (years) [IQR]	38 [32–43]	37 [31–44]	45	38 [31–44]	0.58
Male sex (%)	102 (100)	63 (100)	5 (100)	170 (100)	–
Homosexual (%)	96 (94.1)	63 (100)	5 (100)	164 (96.5)	0.053
Past History of amebiasis (%)	16 (15.7)	9 (14.3)	1 (20.0)	26 (15.3)	0.81
CD4 count (/μl)	262 [98–398]	271 [123–411]	58	269 [107–403]	0.84
HIV-RNA (log copies/ml)	4.60 [3.89–5.32]	4.66 [3.91–5.11]	5.04	4.66 [3.93–5.28]	0.70
AIDS (%)	18 (17.6)	8 (12.7)	2 (40.0)	28 (16.5)	0.40
ART initiated (%)	18 (17.6)	11 (17.5)	1 (20.0)	30 (17.6)	0.98
TPHA test positive (%)	77 (75.5)	40 (63.5)	4 (80.0)	121 (71.2)	0.10
HBV exposure (%)	59 (57.8)	41 (65.1)	2 (40.0)	102 (60.0)	0.36
HCV Antibody positive (%)	3 (2.9)	3 (4.8)	0 (0)	6 (3.5)	0.42

Data are median [interquartile range: IQR] or number (percentage) of patients.
[1]5 cases of perforative peritonitis are included as co-existing diseases. Four cases were diagnosed coincidentally by colonoscopy in asymptomatic patients.
[2]31 cases of colitis, 1 case of perianal abscess, 9 cases of pleuritis, and 2 cases of peritonitis are included as co-existing diseases.
[3]1 case of colitis is included as co-existing diseases.
[4]Chi-square test or non-parametric test was performed for data of colitis and ALA.
UD: undetectable, ART: anti-retroviral therapy, TPHA test: *Treponema pallidum* Hemagglutination Assay test, HBV exposure: HBsAg-positive or HBsAb-positive, and/or HBc-Ab positive.
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agents; 38 cases with promomycin (500 mg t. i. d. for 10 days) and 45 cases with diloxanide furoate (500 mg t. i. d. for 10 days). No significant differences were seen in patients' characteristics, including HIV-1-induced immunocompromised status, serological markers of other STD, and clinical forms and severity of amebiasis between the 83 cases with luminal treatment and 82 cases who did not receive such treatment (Table S1). The median follow-up period after completion of metronidazole or tinidazole treatment was 50 months (inter quartile range: 19–85) in those who received luminal treatment, and 43 months (inter quartile range: 23–98) in those without.

Table 2. Clinical features of amoebic colitis and ALA.

	Colitis (n = 102)	ALA (n = 63)	*P* value
Symptoms			
Diarrhea (%)	71/102 (69.6)	29/63 (46.0)	0.003
Dysentery (%)	57/102 (55.9)	12/63 (19.0)	<0.001
Abdominal pain (%)	23/102 (22.5)	35/63 (55.6)	<0.001
Chest pain (%)	0/102 (0.0)	7/63 (11.1)	<0.001
Peak BT (°C) [IQR][3]	36.8 [36.5–37.4]	39.0 [38.8–39.5]	<0.001
WBC (/μ l) [IQR][3]	5,830 [4490–7580]	11,760 [9460–15170]	<0.001
CRP (mg/dl) [IQR][3]	0.62 [0.16–3.02]	19.15 [10.53–24.75]	<0.001
Frequency of diarrhea[1]			
≤ 5 times/day (%)	63/101 (62.4)	–	
6–10 times (%)	26/101 (25.7)	–	
≥ 11 times (%)	12/101 (11.9)	–	
Size of abscess (mm)	–	59 (10–180)	
Location of abscess[2]			
Right lobe only	–	43/61 (70.5)	
Left lobe only	–	6/61 (9.8)	
Both lobes	–	12/61 (19.7)	
Number of abscesses[1]			
Single (%)	–	45/62 (72.6)	
Multiple (%)	–	17/62 (27.4)	

[1]Data of one case were not available.
[2]Data of two cases were not available.
[3]Data are median [interquartile range: IQR] or number (percentage) of patients.
BT: body temperature, WBC: White Blood Cell counts, CRP: C reactive protein.
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Within the 12-month post-metronidazole treatment period, recurrence of IA was noted in only two patients who did not receive luminal treatment, suggesting reactivation of residual cysts of *E. histolytica* (Figure 1). However, during the entire follow-up period, six in each group experienced recurrence of IA, with no significant difference in the recurrence frequency by the log-rank chi-square test. Multivariate analysis showed that recurrence did not correlate with past history of IA, CD4 count, TPHA, HBV exposure (HBs antigen-positive or HBs antibody-positive), or the presence of extraluminal IA disease (Table 3). However, a positive HCV antibody was significantly associated with IA recurrence. Recurrence also tended to occur in those who acquired new syphilis infection during the follow-up period, though the difference did not reach statistical significance.

Genotypes of *E. histolytica*

Genotyping of *E. histolytica* was performed in samples obtained from 14 patients between December 2009 and March 2010 (colitis, n = 8; ALA, n = 4; colitis and ALA, n = 1; and perianal abscess, n = 1; Table S2). Eleven different genotypes were recognized, including five genotypes (J8, J12, J13, J20, and J23) identified previously in Japan [22], and six newly recognized genotypes (J24-J29). There was no significant relation between *E. histolytica* genotype and clinical presentation.

Discussion

In the present study, retrospective analysis of the medical records of 170 patients with HIV-1-infection and IA showed no

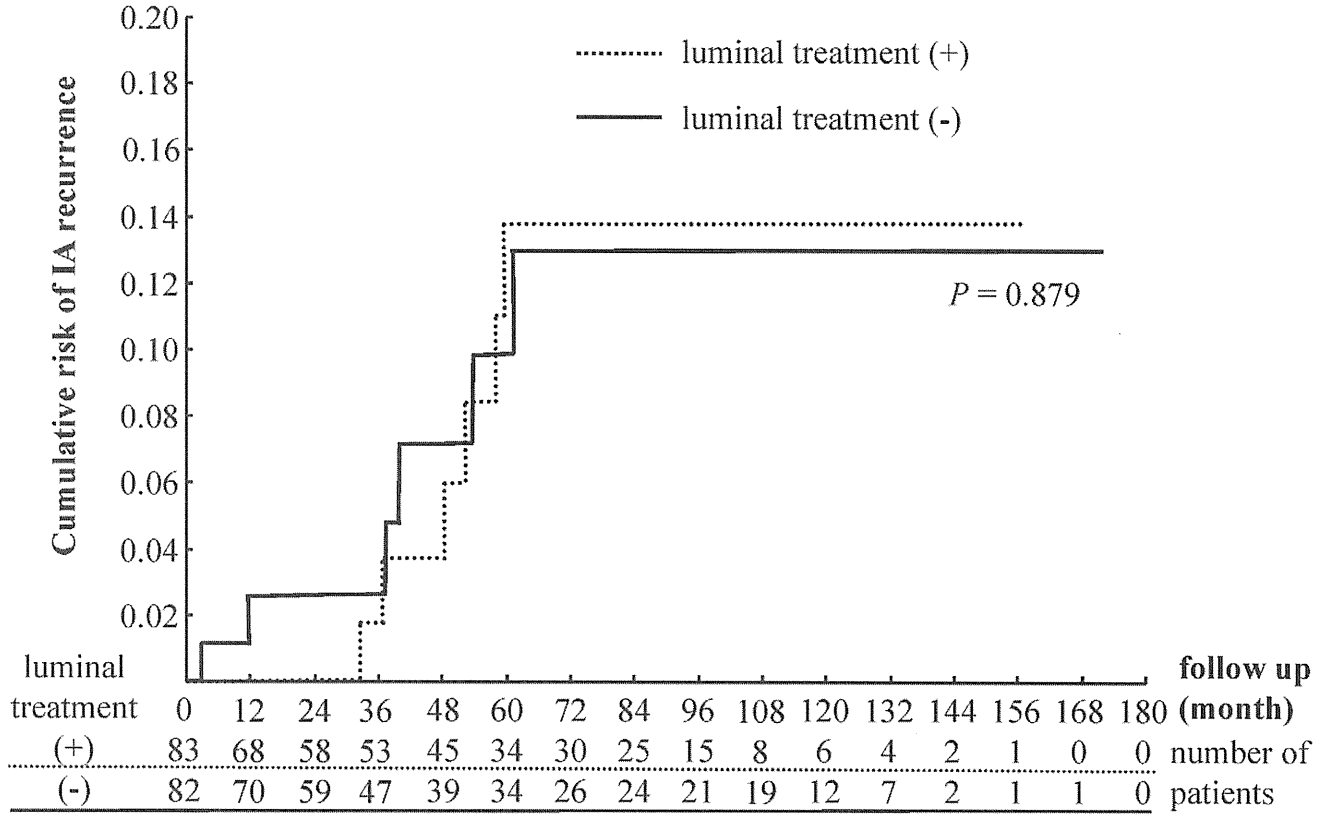

Figure 1. Kaplan-Meier estimates of time to IA recurrence. Cumulative probability of IA recurrence after completion of metronidazole or tinidazole treatment with or without subsequent luminal treatment.
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impact for HIV-1-induced immunocompromised status on the clinical forms of amebiasis. The physical and laboratory findings showed that high fever, leukocytosis and high CRP correlated with extraluminal diseases of amebiasis. In ALA cases, however, leukocyte count correlated positively with CD4 count and negatively with HIV-RNA load, indicating that CRP is more sensitive marker for the detection of the extraluminal diseases in advanced immunocompromised patients.

Only five patients died after the diagnosis of IA; two from IA complications and three from other causes. The results indicate excellent outcome for HIV-1-infected individuals with uncomplicated amebiasis treated with metronidazole or tinidazole, in agreement with previous reports on HIV and non-HIV cases [2,11,12,20,23]. Based on conventional wisdom and written opinion, adequate management of IA should include treatment with a luminal agent following metronidazole or tinidazole treatment, in order to eradicate residual cysts of *E. histolytica* due to the high rate (40–60%) of luminal colonization [2,23–27]. On the other hand, the results of longitudinal observational studies indicated that asymptomatic cyst carriers rarely develop IA, and

Table 3. Multivariate analyses for factors associated with frequency of recurrence.

	No recurrence (n = 153)[1]	Recurrence (n = 12)	Hazard ratio (95.0% CI)	*P* value
Past history of IA[2] (%)	24 (15.7)	2 (16.7)	0.914 (0.186–4.478)	0.911
CD4 counts <200[2] (%)	57 (37.3)	3 (25.0)	0.385 (0.101–1.470)	0.162
TPHA test positive[2] (%)	108 (70.6)	10 (83.3)	2.435 (0.501–11.827)	0.270
HBV exposure[2] (%)	92 (60.1)	7 (58.3)	1.248 (0.364–4.277)	0.725
HCV Antibody positive[2] (%)	3 (2.0)	2 (16.7)	7.664 (1.369–42.890)	0.020
Extraluminal disease[2] (%)	66 (43.1)	4 (33.3)	0.559 (0.163–1.921)	0.356
No luminal agent (%)	76 (49.7)	6 (50.0)	1.070 (0.322–3.559)	0.912
Syphilis during follow-up period (%)	33 (21.6)	7 (58.3)	3.332 (0.961–11.547)	0.059

[1]Five patients died within 6 months from disease onset and their data were excluded from analysis.
[2]Status at diagnosis of IA.
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that cyst form ameba often disappears spontaneously without any treatment [28,29]. There is controversy about the need for cyst eradication following metronidazole or tinidazole treatment, especially in endemic areas where re-infection is frequent. In this study, recurrence of IA within the first year of metronidazole treatment was noted in only two patients of 82 patients who did not receive luminal therapy. Moreover, long-term follow-up indicated IA recurrence also in those who received luminal agents, and the benefits obtained from luminal treatment seemed to have disappeared. IA recurred more frequently in those with HCV infection, which was recently reported to be transmittable sexually among MSM [30], and in those who acquired new syphilis infection during the follow-up period, suggesting that sexually active MSM tend to experience IA recurrence due to re-acquisition of new *E. histolytica* infection. HBV exposure and positive TPHA at IA diagnosis did not correlate with IA recurrence probably because the high prevalence of these two parameters in this study masked the difference between recurrence and non-recurrence cases. Educational approach for safer sex may be more appropriate rather than luminal treatment to prevent IA recurrence after treatment.

Eleven genetic strains of *E. histolytica* were identified in this study and none of them had been reported so far from geographic areas other than Japan [21,22,31,32], indicating that diverse Japan-specific isolates of *E. histolytica* are already prevalent among MSM in Japan. In fact, the *E. histolytica* seropositivity rate in HIV-1-infected MSM in our clinic was as high as 17.9% in 2009 (unpublished data), which is comparable with the seropositivity rate in Japanese MSM reported more than 20 years ago [5]. Unfortunately, we could not compare the genotypes of *E. histolytica* between the incidences of the primary and recurrent IA within the same individuals due to the lack of appropriate stocked samples, which would have probably demonstrated acquisition of new infection.

Considered together, the results emphasize the difficulty of preventing IA recurrence without educational approach to prevent new amebic infection even after successful IA treatment in the high risk groups such as HIV-1-infected MSM. The spread of *E. histolytica* in MSM of other developed countries beyond Asia should be of great concern.

Supporting Information

Table S1 Patient demographics with and without luminal treatment.
(DOC)

Table S2 Genotyping data of 6 STR loci in 14 clinical samples.
(DOC)

Author Contributions

Conceived and designed the experiments: HG JT SO. Performed the experiments: KW AEdC TN. Analyzed the data: KW HG. Contributed reagents/materials/analysis tools: KW HG JT SO. Wrote the paper: KW HG.

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INTERNAL MEDICINE

□ CASE REPORT □

Primary HIV Infection with Acute Transverse Myelitis

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Abstract

Primary HIV infection (PHI) is associated with various neurological disorders. However, acute transverse myelitis (ATM) complicating PHI has not been reported after the introduction of the combination antiretroviral therapy (cART). We encountered one patient with known PHI with clinical presentation of ATM. Treatment with cART and corticosteroids successfully improved symptoms, and no recurrence was noted after discontinuation of cART. In conclusion, concurrent use of cART and corticosteroids was effective against PHI accompanied by ATM and could be withdrawn after improvement of ATM.

Key words: acute transverse myelitis, primary HIV infection, cART

(Intern Med 50: 1615-1617, 2011)
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Introduction

Primary HIV infection (PHI) is associated with various neurological disorders (1, 2). However, PHI complicated with acute transverse myelitis (ATM) had only been reported before the availability of combination antiretroviral therapy (cART) (3). We report a case of ATM, successfully treated with cART and corticosteroids.

Case Report

A 30-year-old homosexual man visited a local hospital with 5-day history of systemic skin eruption, high fever and sore throat. Although these symptoms disappeared spontaneously within one week, PHI was suspected because of the positive result of 4th generation HIV diagnostic test. He was referred to the clinic of our hospital for further management. PHI was confirmed by the negative result of Western blot analysis and high HIV-RNA level (6.38 log copies/mL) with a CD4 count of 601/μL. No treatment for HIV infection was provided because the patient was asymptomatic at that stage. However, he subsequently developed urinary retention and abnormal sensation in the lower limbs, and he returned to the clinic 2 days later. On admission, neurological examination showed normal function of the cranial nerves and no nuchal stiffness. Motor system assessment showed no paresis. The deep tendon reflexes were exaggerated in the lower extremities but normal in the upper extremities. Pathological reflexes such as Babinski reflex and Chaddock sign were not noted. Sensory system examination showed bilateral hypoesthesia and hypalgesia below the level of Th7, and deep sensation was preserved in all extremities. Urinary retention was observed and anal muscle tone was reduced. The cranial and entire spinal magnetic resonance imaging (MRI), with and without gadolinium enhancement, showed no abnormal findings. Examination of the cerebrospinal fluid (CSF) showed mild pleocytosis (cell count: 8.0/μL, mononuclear cells 6.0/μL), a normal protein level (29 mg/dL), and normal IgG index (0.62). The CSF level of myelin basic protein (MBP) was elevated to 1857.3 pg/mL (normal range: <102 pg/mL). Herpes simplex virus, varicella zoster virus, cytomegalovirus and Epstein-Barr virus DNA were negative in the CSF on polymerase chain reaction assay.

ATM was diagnosed by the typical neurological findings with spinal cord inflammation, which was proceeded by PHI. Upon the diagnosis, cART of lopinavir/ritonavir plus abacavir/lamivudine and methylprednisolone pulse treatment (1000 mg for 3 days) were initiated (Fig. 1). The treatment resulted in immediate and rapid improvement of clinical symptoms, and all symptoms disappeared by treatment day 6. The MBP level (less than 31.2 pg/mL) and cell count

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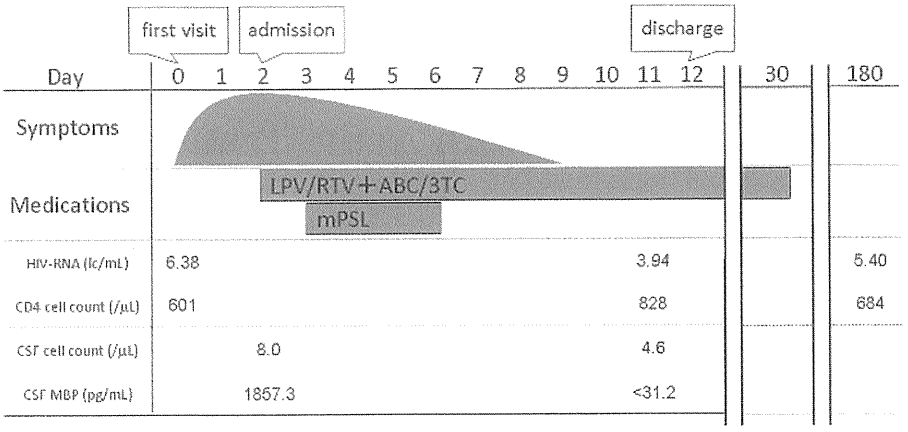

Figure 1. Clinical course. LPV: Lopinavir, RTV: Ritonavir, ABC: Abacabir, 3TC: Lamivudine, mPSL: methylprednisolone, CSF: cerebrospinal fluid, MBP: myelin basic protein

(4.6/μL) in the CSF decreased to the normal ranges at treatment day 9. Methylprednisolone was given for 3 days and cART was continued for one month, which resulted in no relapse of ATM-related symptoms in the subsequent 6 months (Fig. 1), and also no resistant mutation was seen in Protease and Reverse Transcriptase lesions of HIV (data not shown).

Discussion

ATM is a segmental spinal cord injury caused by acute inflammation characterized by acute or subacute motor, sensory, and autonomic (genitourinary and digestive systems) spinal cord dysfunction (4, 5). Although preceding infection is noted in 20-50% of cases (4, 6-9) ATM associated with PHI was only reported before the era of cART (3). There is currently no standard treatment for ATM. Although corticosteroids are the first-line treatment due to the probable mechanisms such as molecular mimicry and the development of autoantibodies, approximately 30 to 50% of patients develop severe sequelae (4, 10, 11).

In the present patient, ATM was diagnosed by the rapid development of symptoms, neurological findings, including clearly-defined bilateral sensory deficits below Th7 level, autonomic dysfunction, and MBP elevation, suggestive of spinal cord inflammation (4, 5). Furthermore, PHI was diagnosed just before the onset of ATM, based on the absence of bands in Western blot analysis and a high titer of HIV-RNA level. It was concluded that PHI was the trigger of ATM.

Although direct cytopathic effects of the virus and immune-mediated toxicity are suggested, pathogenesis of PHI is not fully understood (12-14). There are case reports of rapid improvement of PHI-related symptoms after cART initiation, even though the HIV-RNA level was not completely suppressed at the time of clinical resolution (15, 16). Regarding these phenomena, it is assumed that inhibition of viral replication by cART induces the resolution of symptoms. In the present case, complete recovery was achieved by the combination of steroids and cART (4, 11, 12).

In conclusion, concurrent use of cART and corticosteroids was effective against PHI accompanied by ATM. The immediate improvement in ATM allowed the subsequent discontinuation of treatment.

The authors state that they have no Conflict of Interest (COI).

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