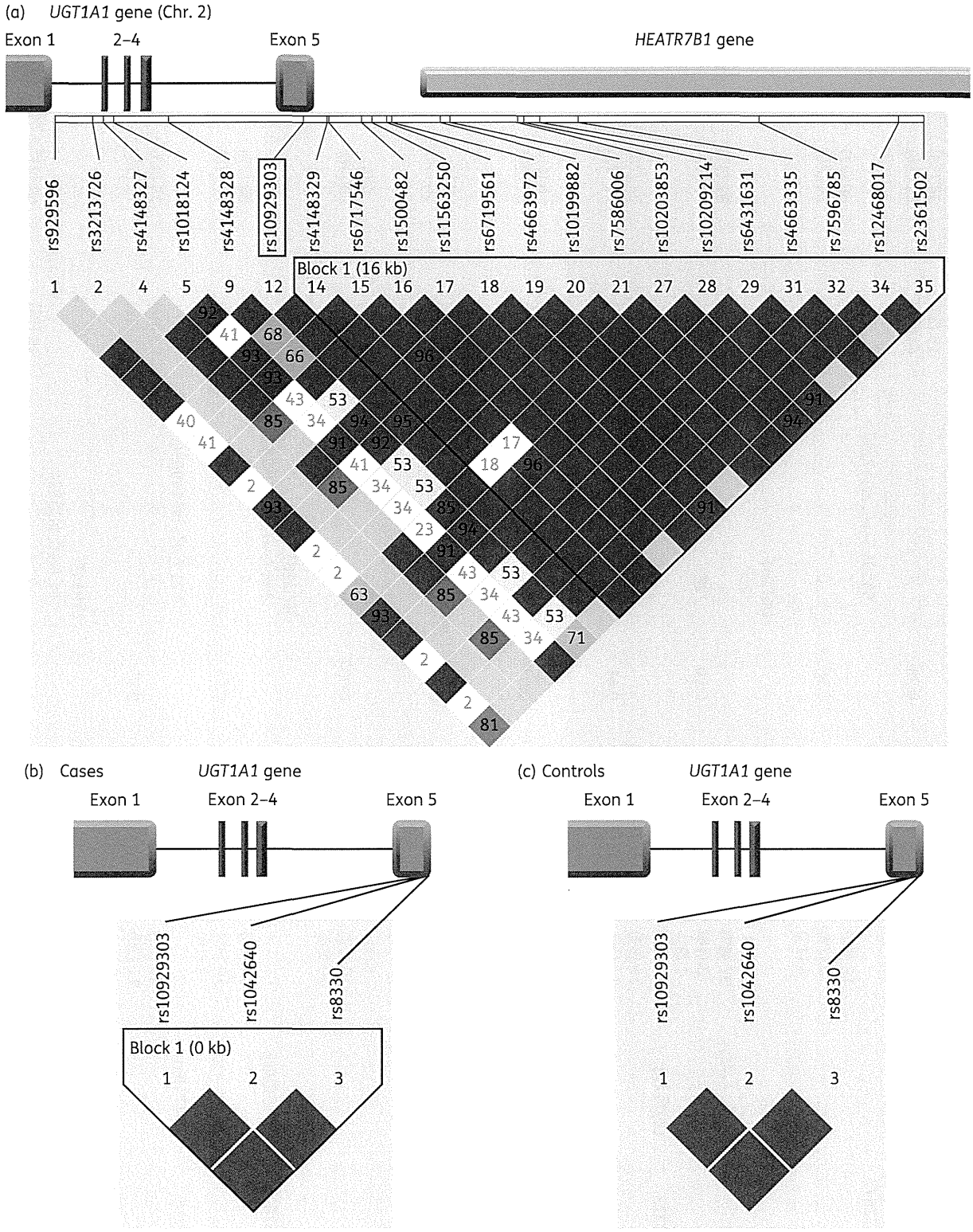

Figure 1. Pairwise linkage disequilibrium analysis of *UGT1A1* and surrounding SNPs. (a) Pairwise linkage disequilibrium analysis of *UGT1A1* and surrounding SNPs using HapMap Japanese samples. SNP c.211 (rs10929303) of the UGT1A-3′-UTR is in tight linkage disequilibrium with the gene next to *UGT1A1* (*HEATR7B1*). Two SNPs at 339 (rs1042640) and 440 (rs8330) of the UGT1A-3′-UTR are not shown in (a), but they are located close to c.211, as shown in (b) and (c). Pairwise linkage disequilibrium analysis of the three risk SNPs in the UGT1A-3′-UTR in (b) 31 cases (patients with atazanavir-induced nephrolithiasis) and (c) 47 controls. The difference between (b) and (c) suggests that the number of risk haplotypes is greater in case patients than in control patients. Estimates of *D′* for SNPs are shown as numbers in the Argyle box. Dark red shading indicates strong linkage disequilibrium (*D′* >0.9). Light blue shading indicates high *D*' values (>0.99) with low statistical significance [LOD (log of the odds) <2].

shows the results of pairwise linkage disequilibrium analysis of *UGT1A1* and SNPs around them derived from HapMap data for the Japanese. On the other hand, there was no difference in the distribution of 16 other SNPs in *ABCB1*, *NR1I2*, *SLCO1B1* and *CYP3A5* between cases and controls. The distribution of UGT1A1*28 was also not different.

Association of genotypes with atazanavir-induced nephrolithiasis

Univariate analysis showed a significant association between atazanavir-induced nephrolithiasis and genotype T/C versus C/C at c.211 (OR=3.8; 95% CI, 1.22–11.6; P=0.022), genotype G/C versus C/C at position 339 (OR=5.9; 95% CI, 1.68–20.9; P=0.006) and genotype G/G or G/C versus C/C at 440 (OR=5.9; 95% CI, 1.68–20.9; P=0.006) of the UGT1A-3′-UTR (Table 3). No other SNPs, including UGT1A1*28, showed any association with nephrolithiasis. Furthermore, basic demographics and established risk factors for nephrolithiasis were not associated with nephrolithiasis, except for infection with hepatitis C virus, which was marginally associated with nephrolithiasis (OR=8.8; 95% CI, 0.98–79.9; P=0.052).

Multivariate analysis adjusted for sex, age and hepatitis C infection identified genotype T/C versus C/C at position c.211 (adjusted OR=3.7; 95% CI, 1.13–11.9; P=0.030), genotype G/C versus C/C at 339 (adjusted OR=5.8; 95% CI, 1.56–21.3; P=0.009) and genotype G/G or G/C versus C/C at 440 (adjusted OR=5.8; 95% CI, 1.56–21.3; P=0.009) of the UGT1A-3′-UTR as independent risk factors for nephrolithiasis (Table 4).

Table 3. Univariate analysis to estimate the association of various factors with atazanavir-induced nephrolithiasis

	OR	95% CI	*P* value
Male	1.7	0.31–9.51	0.53
Age per year	1.0	0.93–1.03	0.39
Weight per 1 kg increment	1.0	0.95–1.03	0.60
BMI per 1 kg/m^2 increment	1.0	0.83–1.11	0.58
CD4 count per 1 cell/mm^3 increment	1.0	1.00–1.00	0.63
Baseline eGFR per 1 mL/min/1.73 m^2 decrement	1.0	0.98–1.03	0.80
HIV-1 viral load per 1 $\log_{10}$/mL increment	0.9	0.62–1.34	0.64
Hepatitis C infection	8.8	0.98–79.9	0.052
Hepatitis B infection	1.5	0.09–25.5	0.77
Treatment naive	0.7	0.25–1.66	0.37
History of nephrolithiasis	3.3	0.57–19.4	0.18
Uric acid per 1 mg/dL increment	1.2	0.93–1.56	0.16
Hypertension	0.7	0.17–3.17	0.68
Diabetes mellitus	0.8	0.07–8.64	0.82
Co-administration of tenofovir	0.7	0.27–1.92	0.51
History of indinavir use	1.6	0.30–8.34	0.60
ABCB1			
193 A/A versus A/G or G/G	0.8	0.32–1.97	0.61
365 T/T versus T/C or C/C	2.5	0.63–10.0	0.19
1236 C/C versus C/T or T/T	0.7	0.22–2.33	0.57
2677 T/T versus T/A or G/G or G/T or G/A or A/A	1.6	0.43–6.12	0.48
3435 T/T versus T/C or C/C	2.1	0.51–8.40	0.31
NR1I2			
131 A/A versus A/C or C/C	1.0	0.40–2.58	0.97
370 G/G versus G/A or A/A	0.7	0.25–1.84	0.44
522 C/C versus C/T or T/T	0.7	0.27–2.04	0.56
1195 C/C versus C/A or A/A	0.7	0.30–2.27	0.70
1232 C/C versus C/T or T/T	0.7	0.25–1.84	0.44
44477 C/C versus C/T or T/T	1.1	0.42–2.67	0.89
63396 C/C versus C/T or T/T	2.2	0.45–10.5	0.33
UGT1A1			
211 G/G versus G/A or A/A	0.9	0.35–2.29	0.82
c.211 T/C versus C/C	3.8	1.22–11.6	0.022
339 G/C versus C/C	5.9	1.68–20.9	0.006
440 G/G or G/C versus C/C	5.9	1.68–20.9	0.006
UGT1A1 *28/*28 or *28/*1 versus *1/*1	2.2	0.45–10.5	0.33
SLCO1B1			
388 G/G versus G/A or A/A	1.6	0.30–8.34	0.60
521 T/T versus T/C or C/C	0.9	0.36–2.43	0.90
CYP3A5			
14 T/T versus T/C or C/C	0.9	0.38–2.33	0.89

Discussion

To our knowledge, this is the first study that has elucidated the association between genetic polymorphisms in the genes encoding proteins that affect atazanavir exposure and atazanavir-induced nephrolithiasis. The results demonstrated that Japanese HIV-1-infected patients who developed atazanavir-induced nephrolithiasis were ~5-fold more likely to have variants in the UGT1A-3′-UTR, compared with those without nephrolithiasis, who were well-matched for other traditional risk factors for nephrolithiasis. These findings suggest a link between genetic factors and nephrolithiasis, a major adverse event of atazanavir that can significantly affect renal function. On the other hand, the results showed no association between variants in *ABCB1* and *SLCO1B1*, the genes that encode drug transporter protein for atazanavir, *CYP3A5*, the main metabolizer of atazanavir, and *NR1I2*, which encodes PXR to regulate the expression of metabolizers and transporters of atazanavir, and atazanavir-induced nephrolithiasis.

This study enrolled only Japanese patients in order to examine a population with comparatively similar genetic backgrounds. It is possible that the association of UGT1A-3′-UTR variants with atazanavir-induced nephrolithiasis could be more significant in people of African or European origin than Japanese or East Asians, considering that the allele frequencies of these variants are higher in these populations according to the HapMap data [e.g. minor allele frequency at position 440 (rs8330): Africans 50%, Europeans 23.3%, Japanese 15.9%, Chinese 15.6%] (www.hapmap.org). Similar studies are needed in these populations to confirm that the association between UGT1A-3′-UTR variants and atazanavir-induced nephrolithiasis is reproducible.

The mechanism by which SNPs in the UGT1A-3′-UTR are associated with the development of nephrolithiasis in patients on an atazanavir-containing regimen is unknown. However, Court

Table 4. Multivariate analysis to estimate the association of SNPs of the UGT1A-3′-UTR with atazanavir-induced nephrolithiasis

UGT1A-3′-UTR	Adjusted OR	95% CI	*P* value
Genotype T/C versus C/C at position c.211	3.7	1.13–11.9	0.030
Genotype G/C versus C/C at position 339	5.8	1.56–21.3	0.009
Genotype G/G or G/C versus C/C at position 440	5.8	1.56–21.3	0.009

Each SNP was tested in the model separately.
Each variable was adjusted for sex, age and hepatitis C infection.

et al.[32] reported that these SNPs are associated with inter-individual variability in acetaminophen (paracetamol) glucuronidation in the human liver, and provide protection against acute liver failure by acetaminophen overdose, probably through more extensive detoxification of acetaminophen via glucuronidation. Because the biotransformation pathways of atazanavir or its metabolites also include glucuronidation,[12] the UGT1A-3′-UTR variants could alter atazanavir metabolism and pharmacokinetics, resulting in increased atazanavir concentration in the blood and increased excretion in urine, facilitating nephrolithiasis formation. Unfortunately, serum and urine concentrations of atazanavir were not measured in the present study. It is also notable that the *UGT1* subfamily has a unique gene structure; the *UGT1* gene has 13 exon 1s from *UGT1A1* to *UGT1A13P*, and exons 2–5, which are common in all mRNAs expressed from the gene.[36] The UGT1A-3′-UTR is located in exon 5, which is commonly present in the UGT1 subfamily (Figure 1), and thus the variants in the UGT1A-3′-UTR might influence not only UGT1A1 but also other UGT1 isoforms that take part in glucuronidation of various substrates,[36] and they might affect atazanavir metabolism and pharmacokinetics as well. Figure 1 also shows that the identified SNPs in the UGT1 3′-UTR are in tight linkage disequilibrium with the gene next to them (*HEATR7B1*), suggesting that the latter could also affect atazanavir metabolism/transportation. To our knowledge, however, there is no information on the role of *HEATR7B1* in drug metabolism/transportation, and the above conjecture remains to be investigated.

In this study, the median serum total bilirubin level in the case patients was higher than that in the control group. Rockwood *et al.*[8] reported a close relationship between hyperbilirubinaemia and the development of atazanavir-induced renal stones. However, no such relationship was found in our previous cohort study.[6] In two pharmacokinetics studies, Rodríguez-Nóvoa *et al.*[20,29] reported that serum bilirubin level correlated with plasma atazanavir concentration, and one can speculate that high bilirubin levels might reflect higher atazanavir concentrations, which result in precipitation of atazanavir in urine and renal stone formation. However, these results are still preliminary and further studies are needed to determine the true relationship between serum bilirubin level and atazanavir-related nephrolithiasis.

Several limitations of this study need to be acknowledged. First, and importantly, although this study identified association between the UGT1A-3′-UTR variants and atazanavir-induced nephrolithiasis, the number of enrolled patients was small in this case–control study; the results need to be interpreted with caution. The results could provide the basis for an exploratory hypothesis and further larger studies are needed to confirm such an association. Second, not all polymorphisms in genes of the targeted proteins were examined. Thus, we might have missed other important SNPs associated with or affecting the metabolism or transportation of atazanavir. There might be other, unknown proteins that take part in the metabolism or transportation of atazanavir that also contribute to susceptibility to atazanavir-induced nephrolithiasis. Third, because renal stone formation occurs as a composite of various factors and the components of nephrolithiasis were not analysed in the study, it is difficult to exclude the effects of classic risk factors for renal stone formation, apart from the genetic factors identified in the present study. However, the two study samples were well matched in terms of risk factors, such as BMI, serum uric acid and history of indinavir use.[4,5,24–26] Furthermore, the susceptibility to nephrolithiasis in patients on an atazanavir/ritonavir-containing regimen is well established; the incidence of nephrolithiasis is 10- to 20-fold higher in patients on atazanavir/ritonavir-containing ART than in patients on other protease inhibitor-containing ART regimens.[6,7] Fourth, because functional data are not yet available, clinical or biochemical studies to confirm the results obtained here are certainly needed. We did not measure atazanavir concentration in blood or urine.

In conclusion, in a setting where other predisposing factors for nephrolithiasis were well matched, the present study demonstrates that the Japanese HIV-1-infected patients who developed atazanavir-induced nephrolithiasis were ~5-fold more likely to have variants in the UGT1A-3′-UTR compared with those without nephrolithiasis. Further studies are warranted to confirm this association and to elucidate how these SNPs might influence the metabolism and excretion of atazanavir and the formation of nephrolithiasis.

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Pharmaceutical, Co., Roche Diagnostics K.K. and ViiV Healthcare, Co. All other authors: none to declare.

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Brain Magnetic Resonance Imaging Screening Is Not Useful for HIV-1-Infected Patients Without Neurological Symptoms

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Abstract

We investigated the diagnostic usefulness of brain magnetic resonance imaging (MRI) screening in HIV-1-infected patients without neurological symptoms in detecting intracranial diseases at early stages. In this retrospective analysis, the study patients were HIV-1-infected patients who underwent brain MRI scan in clinical practice between 2001 and 2013. We excluded patients with MRI for (1) follow-up examination for prediagnosed intracranial diseases, (2) cancer staging, (3) screening mycobacterium/bacteria/fungi disease proliferation in the brain, and (4) evaluation for meningitis/encephalitis. The study patients ($n=485$) were classified into two groups: those who underwent brain MRI scan without any neurological symptoms/signs (asymptomatic patients, $n=158$) and those who underwent MRI due to such symptoms (symptomatic patients, $n=327$). Asymptomatic patients had lower CD4 counts than symptomatic patients (median 78 versus 241/μl). Intracranial diseases were detected in three (2%) of the asymptomatic patients [two toxoplasmosis and one progressive multifocal leukoencephalopathy (PML)] compared to 58 (19%) of the symptomatic patients (the χ^2 test, $p<0.01$). The latter included toxoplasmosis ($n=10$), PML ($n=7$), cytomegalovirus encephalitis ($n=3$), primary central nervous system lymphoma ($n=3$), cryptococcoma/meningitis ($n=3$), and HIV-associated dementia ($n=17$). Among symptomatic patients, intracranial diseases were common in those with slurred speech (3/6, 50%), seizure (4/10, 40%), eyesight/vision abnormality (5/16, 31%), altered mental status (8/31, 26%), and hemiplegia/numbness (13/50, 26%). For patients with CD4 count $<200/\mu$l, intracranial diseases were detected in only 3 (3%) of 144 asymptomatic patients, compared with 46 (32%) of 113 symptomatic patients ($p<0.01$). Brain MRI screening for HIV-1-infected patients without neurological symptoms is of little value.

Introduction

PATIENTS WITH ADVANCED HIV-1 INFECTION are prone to develop intracranial opportunistic diseases, such as toxoplasma encephalitis, primary central nervous system lymphoma (PCNSL), progressive multifocal leukoencephalopathy (PML), and cytomegalovirus (CMV) encephalitis.[1] Although the introduction of antiretroviral therapy (ART) substantially decreased the incidence of neurological opportunistic infections,[2,3] such diseases have high associated mortality even with appropriate treatment, and recurrences and residual neurological deficits can occur.[4,5] Because delayed diagnosis of these intracranial diseases has a detrimental effect on patients with HIV-1 infection,[5,6] early diagnosis, not to mention prevention, of such diseases is of importance.

Brain magnetic resonance imaging (MRI) is often preferred to computed tomography (CT) in establishing the diagnosis of many of these diseases due to its superior sensitivity to subtle white matter and meningeal disease.[7–10] However, there is no information on the utility of brain MRI screening for HIV-1-infected patients without neurological symptoms/signs in detecting intracranial opportunistic diseases at early stages. This observational study was designed to assess the usefulness of brain MRI screening of such patients with HIV-1 infection.

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Materials and Methods

Study design, setting, and participants

We conducted an observational single-center study to investigate the usefulness of brain MRI screening in HIV-1-infected patients without neurological symptoms who warrant investigation for intracranial diseases. The study was conducted at the AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo, the largest referral center for HIV care in Japan.[11] The study patients were those who fulfilled the following inclusion criteria: HIV-1-infected patients who underwent brain MRI scan in clinical practice between June 2001 and August 2013. In addition, the following exclusion criteria were applied: patients who underwent brain MRI for (1) follow-up examination during the study period because of intracranial diseases such as opportunistic infections, stroke, or malignancy, which were diagnosed prior to the referral to our clinic, (2) staging of malignant tumors, (3) screening mycobacterium/bacteria/fungi disease proliferation in the brain in patients who were already diagnosed with mycobacterial diseases or bacteremia or fungemia, and (4) evaluation of meningitis/encephalitis.

The study patients ($n=485$) were classified into those who underwent brain MRI scan without any neurological symptoms, such as seizure, altered mental status, hemiplegia/numbness, headache, or fever (asymptomatic patients, $n=158$), and those who underwent MRI due to the abovementioned symptoms, which can suggest a focal brain lesion[5] (symptomatic patients, $n=327$). Asymptomatic patients included those who underwent MRI due to positive antitoxoplasma IgG antibody ($n=38$) and positive serum cryptococcal antigen ($n=1$). At our clinic, patients with a low CD4 cell count (typically less than 200/μl) often underwent brain MRI even though they had no neurological symptoms/signs that would warrant a brain imaging examination to rule out intracranial opportunistic infections or malignancy at early stages.

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

Measurements

At our hospital, brain MRI was routinely read by one experienced radiologist and the findings were confirmed by another radiologist. Furthermore, the MRI diagnosis was confirmed by reviewing the medical records and follow-up brain imaging when available. The diagnostic criteria for cryptococcal meningitis, cytomegalovirus encephalitis, and toxoplasmic encephalitis were those adopted by the AIDS Clinical Trials Group (ACTG)-A5164.[12] HIV-associated dementia in this study was diagnosed based on the MRI findings, which included generalized atrophy and prominent white matter changes plus cognitive impairment based on the chart review, and not necessarily required neurocognitive function tests.[8] The reasons for conducting an MRI were also extracted from the medical records. Baseline characteristics and HIV-1-related variables at the time of brain MRI were also extracted from the medical records. They included age, sex, ethnicity, history of AIDS, route of HIV-1 transmission, treatment status for HIV-1 infection (either treatment naive or experienced), CD4 cell count, and HIV viral load. For CD4 count and HIV load, we used data collected closest to and preceding by up to 3 months the day of the brain MRI. In Japan, because the prescription period under the health care system is limited to 3 months, patients need to visit the HIV Clinic at least once every 3 months for prescriptions as well as monitoring CD4 cell count and HIV-1 load.[11]

Statistical analysis

Baseline characteristics were compared between asymptomatic and symptomatic patients using the Student's t-test and χ^2 test (Fisher's exact test) for continuous and categorical variables, respectively. Prevalence of intracranial diseases was calculated among asymptomatic patients and compared to that of symptomatic patients with the χ^2 test. The logistic regression model was used to estimate the associations of lack of neurological symptoms/signs over the presence of such symptoms/signs with the MRI findings of intracranial diseases. The model was adjusted for age, sex, CD4 count, HIV treatment status, and history of AIDS. Subgroup analysis included the prevalence of intracranial diseases in patients with a CD4 count $<200/\mu$l. Statistical significance was defined as two-sided p values <0.05. We used odds ratios (ORs) with 95% confidence intervals (95% CIs). All statistical analyses were performed with The Statistical Package for Social Sciences ver. 21.0 (SPSS, Chicago, IL).

Results

The study included 485 patients who underwent a brain MRI scan in clinical practice, of whom 158 had no neurological symptoms (asymptomatic) and 327 did have such symptoms (symptomatic). Of the total patients, 475 (98%) were Asians, 446 (92%) were males, and 365 (75%) were infected with HIV-1 through homosexual contact (Table 1). The median age of the study patients was 41 [interquartile range (IQR) 34–51]. Asymptomatic patients had a lower CD4 count [median 78/μl, interquartile range (IQR) 21–237, symptomatic: 241/μl, 60–470 ($p<0.01$)] and higher HIV-1 viral load [4.84 $\log_{10}$/ml, IQR 2.97–5.62, symptomatic: 2.95 $\log_{10}$/ml, 1.70–5.11 ($p<0.01$)] than symptomatic patients. Asymptomatic patients were more likely to be treatment naive (68% versus 41%, $p<0.01$) and have a history of AIDS (62% versus 47%, $p<0.01$). There was no significant difference in other baseline characteristics between the two groups (Table 1).

Among the 158 asymptomatic patients, brain MRI screening detected toxoplasmosis ($n=2$) and PML ($n=1$, with CD4 43/μl), i.e., a prevalence of intracranial diseases of 2%. The two patients with toxoplasmosis underwent brain MRI due to positive antitoxoplasma IgG antibody with a titer of 20,480 (CD4 168/μl) and 1,280 (CD4 16/μl) IU/ml. In asymptomatic patients who underwent brain MRI due to positive antitoxoplasma IgG antibody, intracranial diseases were detected in 3 (8%) out of 38 patients (Table 2). On the other hand, brain MRI for symptomatic patients detected 58 intracranial diseases with a prevalence of 19%. The cases included toxoplasmic encephalitis ($n=10$), PML ($n=7$), CMV encephalitis ($n=3$), PCNSL ($n=3$), cryptococcosis/meningitis ($n=3$), herpes simplex virus encephalitis ($n=1$), HIV-associated dementia ($n=17$), acute cerebral infarction ($n=8$), gummatous

TABLE 1. CLINICAL CHARACTERISTICS OF THE STUDY PATIENTS ACCORDING TO NEUROLOGICAL SYMPTOMS

	All patients (n=485)	*Patients without neurological symptoms (n=158)*	*Patients with neurological symptoms (n=327)*	p *value*
Male sex, *n* (%)	446 (92)	146 (92)	300 (92)	0.86
Age[†]	41 (34–51)	42 (33–52)	41 (35–49)	0.95
Asian, *n* (%)	475 (98)	154 (98)	321 (98)	0.74
CD4 cell count (/μl)[a]	178 (41–420)	78 (21–237)	241 (60–470)	<0.01
HIV-1 load (log_{10}/ml)[a]	4.20 (1.70–5.26)	4.84 (2.97–5.61)	2.95 (1.70–5.11)[b]	<0.01
Homosexual contact, *n* (%)	364 (75)	117 (74)	247 (76)	0.74
Treatment naive, *n* (%)	240 (50)	107 (68)	133 (41)	<0.01
History of AIDS, *n* (%)	250 (52)	98 (62)	152 (47)	<0.01

[a]Median (interquartile range).
[b]Data on HIV-1 load are not available for two patients.

syphilis ($n=1$), tuberculoma ($n=1$), metastatic cancer ($n=1$), chronic subdural hematoma ($n=1$), schwannoma ($n=1$), and progressive supranuclear palsy ($n=1$) (Table 2). In asymptomatic patients, intracranial diseases were less likely to be detected by brain MRI, compared to symptomatic patients [by univariate and multivariate analysis (OR=0.1; 95% CI, 0.03–0.29; $p<0.01$) (adjusted OR=0.1; 95% CI, 0.02–0.17; $p<0.01$)]. Patients with higher CD4 counts were also less likely to have intracranial diseases (per 100/μl increment, adjusted OR=0.7; 95% CI, 0.55–0.83; $p<0.01$). Among the symptomatic patients, those who presented with slurred speech, seizure, eyesight/vision abnormality, altered mental status, and hemiplegia/numbness were highly likely to have intracranial diseases, with a prevalence of 50%, 40%, 31%, 26%, and 26%, respectively (Table 3).

Subgroup analysis limited to data of patients with CD4 count of <200/μl showed that the abovementioned three intracranial diseases were detected in 144 asymptomatic patients with a prevalence of 3%, compared to 46 (32%) of 113 symptomatic patients (asymptomatic over symptomatic, OR=0.1; 95% CI, 0.02–0.19; $p<0.01$) (Table 2). Only a few intracranial opportunistic diseases were diagnosed in patients with a CD4 count of ≥200/μl; PCNSL ($n=1$), HIV-associated dementia ($n=4$), acute cerebral infarction ($n=6$), metastatic cancer ($n=1$), and progressive supranuclear palsy ($n=1$).

Discussion

In this observational study of patients who underwent brain MRI screening in clinical practice, only 2% of patients without neurological symptoms/signs that warranted investigation of intracranial diseases were found to have intracranial diseases, whereas a significantly higher prevalence (19%) of intracranial diseases was detected in patients who underwent brain MRI due to such symptoms. Among patients with a CD4 count of <200/μl, who are reported to be at high risk for intracranial diseases,[5,10] the result was similar; 3% and 32% of asymptomatic and symptomatic patients, respectively, were found to have intracranial diseases. On the other hand, high detection rates of intracranial diseases by brain MRI were observed in patients who presented with slurred speech (50%), seizure (40%), eyesight/vision abnormality (31%), altered mental status (26%), and hemiplegia/

TABLE 2. PREVALENCE OF INTRACRANIAL DISEASES DETECTED BY BRAIN MAGNETIC RESONANCE IMAGING ACCORDING TO NEUROLOGICAL SYMPTOMS

Intracranial diseases	*Patients without neurological symptoms (n=158)*	*Patients without neurological symptoms with CD4 <200/μl (n=144)*	*Patients with neurological symptoms (n=327)*	*Patients with neurological symptoms with CD4 <200/μl (n=113)*	*Positive toxoplasma Ab and without neurological symptoms (n=38)*
Toxoplasmosis	2 (1)	2 (2)	10 (3)	10 (7)	2 (1)
PML	1 (1)	1 (1)	7 (2)	7 (5)	1 (1)
HIV-associated dementia			17 (6)	13 (9)	
Malignant lymphoma			4 (1)	3 (2)	
CMV encephalopathy			3 (1)	3 (2)	
Cryptococcoma/meningitis			3 (1)	3 (1)	
HSV encephalopathy			1	1	
Gummatous syphilis			1	1	
Tuberculoma			1	1	
Metastatic cancer			1		
Cerebral infarction			8 (3)	2 (1)	
Others			3 (1)	2 (1)	
Total	3 (2)	3 (3)	59 (19)	46 (32)	3 (8)

Data are numbers (percentages) of patients.
Ab, antibody; PML, progressive multifocal leukoencephalopathy; CMV, cytomegalovirus; HSV, herpes simplex virus.

TABLE 3. PREVALENCE OF INTRACRANIAL DISEASES DETECTED BY BRAIN MAGNETIC RESONANCE IMAGING ACCORDING TO NEUROLOGICAL SYMPTOM CATEGORIES

	Intracranial diseases	*Prevalence of intracranial diseases*
Slurred speech ($n=6$)	Cerebral infarction $n=2$ PML $n=1$	50%
Seizure ($n=10$)	Toxoplasmosis $n=2$ PML $n=1$ HSV encephalitis $n=1$	40%
Eyesight/vision abnormality ($n=16$)	Malignant lymphoma $n=2$ HIV-associated dementia $n=2$ Metastatic cancer $n=1$	31%
Altered mental status ($n=31$)	Toxoplasmosis $n=2$ HIV-associated dementia $n=2$ Cryptococcoma/meningitis $n=2$ PML $n=1$ Tuberculoma $n=1$	26%
Hemiplegia/numbness ($n=50$)	Cerebral infarction $n=5$ Toxoplasmosis $n=3$ PML $n=3$ HIV-associated dementia $n=1$ Other $n=1$	26%
Neurocognitive impairment ($n=62$)	HIV-associated dementia $n=9$ Cerebral infarction $n=1$ CMV encephalitis $n=2$	19%
Fever work-up ($n=12$)	Malignant lymphoma $n=1$ HIV-associated dementia $n=1$	17%
Dizziness/vertigo/tinnitus ($n=45$)	Toxoplasmosis $n=1$ PML $n=1$ Malignant lymphoma $n=1$ HIV-associated dementia $n=1$ CMV encephalitis $n=1$	11%
Abnormal ophthalmologic examination ($n=11$)	HIV-associated dementia $n=1$	9%
Headache ($n=49$)	Toxoplasmosis $n=2$	4%
Syncope ($n=16$)		0%

PML, progressive multifocal leukoencephalopathy; HSV, herpes simplex virus; CMV, cytomegalovirus.

numbness (26%). The present study indicates that brain MRI screening for HIV-1-infected patients without neurological symptoms/signs, even those with a low CD4 count (< 200/μl), is of little value. In contrast, MRI screening is useful for patients with particular neurological symptoms/signs. These findings can help reduce unnecessary brain MRI examinations and can be helpful in clinical decision making.

Interestingly, in both of the two asymptomatic toxoplasmic encephalitis patients who underwent brain MRI screening because of positive antitoxoplasma IgG antibody, the antibody titer was very high (20,480 IU/ml and 1,280). Together with the fact that the prevalence of intracranial diseases in asymptomatic patients with positive antitoxoplasma IgG antibody was higher (8%) than the 2% in the entire group of asymptomatic patients, brain MRI screening for patients without neurological symptoms/signs who presented with high antitoxoplasma antibody may be of value and clinically justifiable.

Our study has certain limitations. First, because brain MRI was performed at the discretion of the treating physician, patient selection bias, especially among those without neurological symptoms/signs, cannot be ruled out. However, we had a large number of study patients, and considering the availability and cost of an MRI scan, the results of the present study are of value and are useful in clinical decision making. Second, because endemic opportunistic infections vary depending on the region[13,14] and the majority of our patients were Asian, the results of the present study might not be applicable to patients in other regions. Third, in this study the diagnosis of HIV-associated dementia was based on the MRI findings plus cognitive impairment based on a chart review, and the patients did not necessarily undergo neurocognitive function tests.[8] This is because the present study included patients from 2001, long before the diagnostic Frascati criteria for an HIV-associated neurocognitive disorder that required neurocognitive function tests were established.[15]

In conclusion, although our results suggest that brain MRI screening is of little value in HIV-1-infected patients without neurological symptoms/signs that warrant investigation on intracranial diseases, it should be performed in HIV-1-infected patients who present with particular neurological symptoms, such as slurred speech and seizure.

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Author Disclosure Statement

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Short Report: Asymptomatic Intestinal Amebiasis in Japanese HIV-1–Infected Individuals

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Abstract. Seventy-one asymptomatic human immunodeficiency virus-1 (HIV-1) -infected individuals who underwent colonoscopy for detection of diseases other than amebiasis were included in this study. Ulcerative lesions caused by *Entamoeba histolytica* were identified by colonoscopy and biopsy in 11.3% (8 of 71) of individuals. Stool microscopic examination hardly identified *Entamoeba*, whereas serum antibody against *E. histolytica* was often elevated in patients with subclinical intestinal amebiasis. Human leukocyte antigen (HLA) class II allele against *E. histolytica* infection (DQB1*06:01) was frequently identified in these patients. This study emphasizes the endemic nature of *E. histolytica* infection in our cohort and the difficulties in epidemiological control.

INTRODUCTION

Invasive amebiasis caused by *Entamoeba histolytica* is the second most common cause of parasite infection-related mortality worldwide, accounting for 40,600 to 73,800 deaths annually.[1] Recent studies indicated that invasive amebiasis is prevalent in not only developing countries, where food or water is contaminated with stool, but also, East Asian developed countries, including Japan, as a sexually transmitted infection.[2–5] We reported previously high seropositivity for *E. histolytica* among asymptomatic human immunodeficiency virus-1 (HIV-1)-infected individuals in Japan and showed relatively high incidence of invasive amebiasis in that population, probably because of exacerbation of subclinical infection.[6] Other groups also reported that serum antibody against *E. histolytica* can be elevated, even in asymptomatic-infected individuals, and that seroconversion was seen in the absence of any symptoms in longitudinal follow-up in endemic areas.[7] These results indicate that subclinical infection of *E. histolytica* is frequent in high-risk populations, making it difficult to control *E. histolytica* endemicity.

Evidence suggests that human leukocyte antigen (HLA) type plays a role in amebiasis. For example, Duggal and others[8] reported previously that HLA DQB1*0601 seemed to provide protection against *E. histolytica* infection in Bangladeshi children.

This cross-sectional study was designed to determine the prevalence of ulcerative lesions associated with *E. histolytica* infection in asymptomatic HIV-1–infected individuals in Japan. We also examined the pathogenesis of subclinical intestinal amebiasis and the role of HLA genotypes.

MATERIALS AND METHODS

Ethics statement. The study was approved by the Human Research Ethics Committee of our hospital, the National Center for Global Health and Medicine in Tokyo. The study was conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all participants. No children were included in the study.

Study design and participants. This cross-sectional study included HIV-infected patients who underwent colonoscopy between June of 2010 and June of 2013. One week before colonoscopy, each patient filled out a questionnaire about lower gastrointestinal symptoms based on the Gastrointestinal Symptom Rating Scale (GSRS) rating on a seven-graded Likert scale.[9] Asymptomate for lower gastrointestinal diseases was defined as GSRS scores of one or two for three questions on the diarrhea syndrome domain (diarrhea, loose stools, and urgent need to defecate) and one question on bloody stool.[10] Serum antibody testing against *E. histolytica* was performed in all participants on the day of colonoscopy. Serum antibody was tested by indirect fluorescent antibody assay using whole *E. histolytica* antigen according to the protocol described in the instruction sheet of the approved kit (bioMerieux, SA). Seropositivity was defined as positive response in a serum sample diluted at 1:100 ($\times 100$), and anti-Eh titer was determined by the highest dilution for the positive response. HLA type was determined by standard sequence-based genotyping (HLA Laboratory, Kyoto, Japan). The diagnosis of subclinical intestinal infection of *E. histolytica* was established on confirmation of one or two of the following two criteria: (1) identification of amebic trophozoites in biopsy specimens from gross ulcerative lesions obtained during colonoscopy and/or (2) no pathogens identified in biopsy specimens of gross ulcerative lesion, which were compatible with amebic ulcer,[11] but ulcerative lesion resolved completely after metronidazole monotherapy as confirmed by colonoscopy.

Statistical analysis. The patients' characteristics and serum positivities for anti-*E. histolytica* antibody were compared using χ^2 or Mann–Whitney U test for qualitative or quantitative variables, respectively. Statistical significance was defined as two-sided P value < 0.05. All statistical analyses were performed using The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL).

RESULTS

Study population. In total, 380 HIV-1–infected individuals were enrolled during the study period, and 71 patients met the criteria of no symptoms for lower gastrointestinal diseases according to the GSRS. The most common reason for colonoscopy was colorectal cancer screening ($N = 48$), whereas

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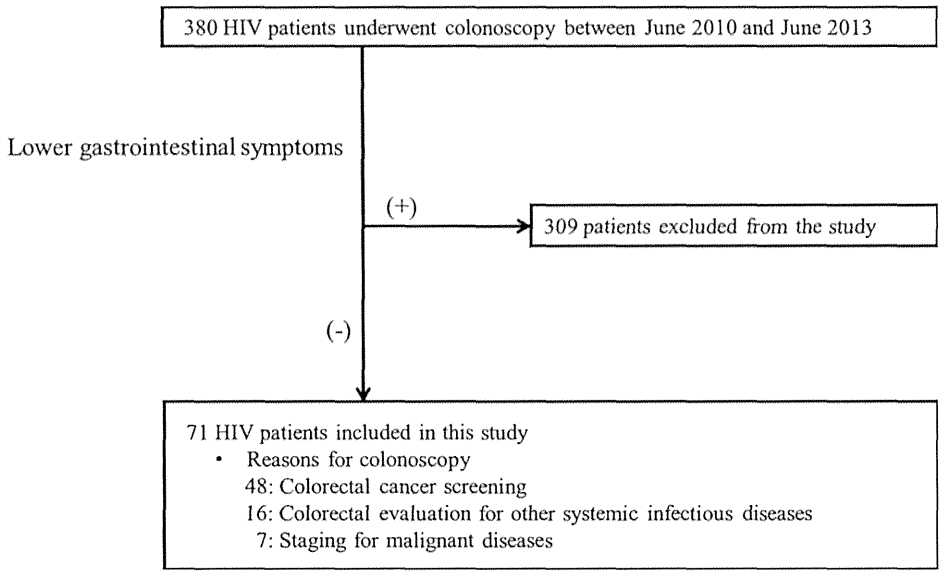

FIGURE 1. Flow diagram of the patient recruitment process. Lower abdominal symptoms were collected based on the GSRS rating on a seven-graded Likert scale at 1 week before colonoscopy.

the other 23 patients underwent colonoscopy for evaluation of progression of malignancies or infections (e.g., malignant lymphoma, Kaposi's sarcoma, tuberculosis, and cytomegalovirus) (Figure 1).

Frequency of intestinal amebic infection among asymptomatic HIV-1–infected individuals. Amebic colitis was confirmed in eight (11.3%) cases. Gross ulcerative lesions were identified by colonoscopy in all eight cases. Amebic trophozoite was identified in the biopsy specimens of five cases (Figure 2). Although amebic trophozoites were not identified in the biopsy specimens of the other three cases, their sera were positive for antibody against *E. histolytica*. In all patients, the ulcerative lesions resolved completely after metronidazole monotherapy.

Clinical features and presentation of patients with and without intestinal amebic infection. As shown in Table 1, patients with amebic intestinal ulcerative lesions tended to be younger, be male homosexuals, have low CD4 counts, and have high HIV-RNA levels, although these differences were not statistically significant. Multiple ulcerative lesions were found in four cases (50%), and the most frequently involved location was the cecum (five cases; 62.5%). Serum antibody against *E. histolytica* was positive in 7 of 8 (87.5%) patients with amebic intestinal ulcerative lesions compared with positivity in only 11 of 63 (17.5%) patients without amebic ulcerative lesions (Table 2).

From the limited data on fecal occult blood testing (FOB) and stool microscopic examination before treatment in cases with amebic ulcerative lesions, FOB was positive in two of three cases (66.7%), and the cyst form, not trophozoite form, *Entoamoeba* was found in only one of four cases (25%).

HLA class II allele frequencies in patients with and without subclinical intestinal amebiasis. HLA data were available for 57 patients (7 of 8 patients with amebiasis and 50 of 63 patients without amebiasis) in our study. We investigated the relation between HLA alleles identified in more than five patients (frequency > 10%) and subclinical intestinal amebiasis. HLA DQB1*06:01 allele was significantly more frequent in patients with subclinical intestinal amebiasis than those without it (Table 3). All the HLA DQB1*06:01 holders were heterozygotes. The frequency of the HLA DRB1*15:02 allele was also significantly higher in patients with subclinical intestinal amebiasis ($P = 0.05$); 7 of 10 patients with HLA DQB1*06:01 also held HLA DRB1*15:02. No colonic amebic ulceration was detected in DQB1*06:01 (–)/DRB1*15:02 (+) patients. Thus, DQB1*06:01 seemed to be the primary HLA allele associated with subclinical intestinal amebiasis in the study population.

DISCUSSION

The pathogenesis of amebiasis remains unclear, including the incubation period after cyst ingestion and the mechanism of spontaneous remission. We reported previously high seroprevalence of *E. histolytica* (21.3%) in HIV-1–infected individuals and that the majority of these patients (78.3%) had no history of invasive amebiasis. In that study, the patients were considered to be at high risk for developing symptomatic amebic infection in longitudinal follow-up (about 20% within the first 1 year of the follow-up period).[6] Based on those results, we speculated the presence of subclinical intestinal amebiasis in patients positive for antibody against *E. histolytica* in the serum resulting in high frequency of symptomatic amebic diseases thereafter, although we did not identify the lesions of *E. histolytica* in these individuals in that study. However, Okamoto and others[12] reported that intestinal ulcerative lesions of *E. histolytica* were rare based on colonoscopic examination in the general population in Japan with positive FOB (0.1%; 4 of 5,193). Our group reported previously that patients with cecal amebic ulcers were sometimes asymptomatic.[11] In this regard, however, the clinical significance of *E. histolytica* infection in asymptomatic individuals had not been fully assessed. In this study, we identified gross amebic ulcers by colonoscopy in 11.2% of asymptomatic HIV-1–infected individuals.

Detection of intestinal amebiasis in asymptomatic individuals is important for not only treatment but also, epidemiological control, especially in endemic areas, because individuals

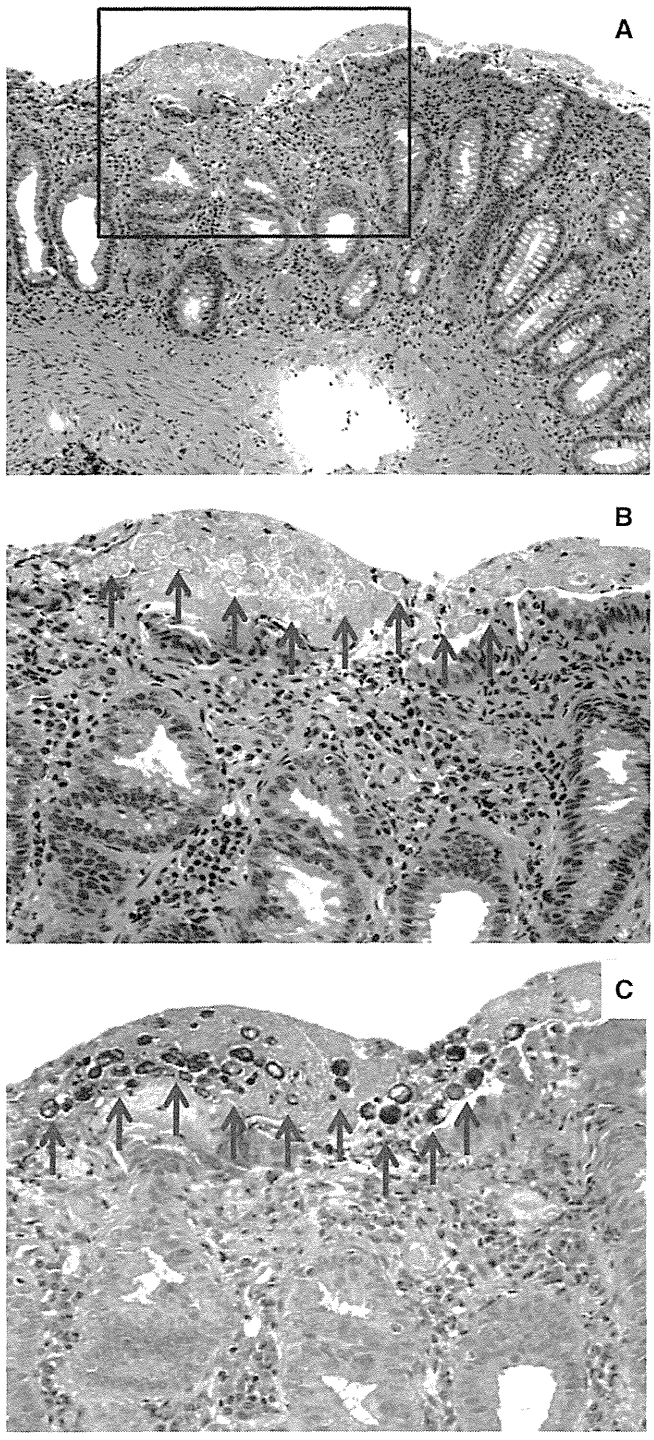

FIGURE 2. Histopathological findings in subclinical intestinal amebiasis. Colonic tissue section was obtained during colonoscopy from a representative asymptomatic patient. *E. histolytica* on the surface of large-intestinal mucosa was clearly stained with periodic acid-Schiff (PAS) staining (green arrows). (**A**) Hematoxylin-eosin staining, ×100. (**B**) Higher magnification of the boxed area in **A**. Hematoxylin-eosin staining, ×400. (**C**) PAS staining, ×400.

with intestinal amebic ulcers can act as a reservoir for *E. histolytica*. However, it is sometimes difficult to identify amebiasis in these individuals, because they lack typical abdominal symptoms related to amebiasis, such as tenesmus, diarrhea, and dysentery. Moreover, our results showed that stool microscopic examination hardly identified amebiasis in these individuals. FOB is more sensitive than stool microscopic examination. However, FOB was positive in 72.7% (16 of 22) of patients free of amebic ulceration. Serum antibody against *E. histolytica* might be a sensitive marker of amebic ulcer in asymptomatic individuals. However, low titers of serum antibody were frequently found in individuals without amebic ulcer. The optimal cutoff value of antibody titer for amebic ulcer is still unclear (for cutoff titer of ×100, sensitivity is 87.5%, and specificity is 82.5%, whereas for cutoff titer ×400, sensitivity is 75.0%, and specificity is 95.2%) (Table 2).

Interestingly, our analysis showed high frequency of HLA DQB1*06:01 heterozygote in patients with subclinical intestinal amebiasis. This allele was reported previously to provide protection against *E. histolytica* infection in Bangladeshi patients.[8] One possible explanation is that ulcerative lesions could occur asymptomatically in patients with HLA DQB1*06:01 and that their immune system could prevent the development of invasive disease from *E. histolytica*, resulting in the high frequency of subclinical intestinal amebiasis observed in our cross-sectional analysis. Genetic differences between Bangladeshi and Japanese patients should also be considered. HLA DQB1*06:01 and DRB1*15:01 were the most common haplotypes in Bangladesh, although they were not identified in our patients.

TABLE 1
Characteristics of patients with and without subclinical intestinal amebiasis

	Amebiasis	No amebiasis	*P* value
n	8	63	
Age (years), median (range)	39 (27–62)	51 (26–81)	0.07
Male sex (%)	8/8 (100%)	56/63 (88.9%)	1.00
Men who have sex with men (%)	8/8 (100%)	44/63 (69.8%)	0.10
Past history of amebiasis (%)	0/8 (0%)	7/63 (11.1%)	1.00
CD4/μL, median (range)	301 (70–584)	436 (21–1,697)	0.28
HIV-RNA (LC/mL), median (range)	4.02 (UD–5.41)	UD (UD–5.85)	0.09

LC/mL = log 10 copies per milliliter; UD = undetectable.

TABLE 2
Clinical presentation of patients with and without subclinical intestinal amebiasis

	Amebiasis	No amebiasis	*P* value
n	8	63	
Serum positivity for anti-*E. histolytica* antibody (%)	7/8 (87.5%)	11/63 (17.5%)	< 0.001
< ×100	1	52	
×100	1	5	
×200	0	3	
×400	3	2	
×800	1	1	
×1,600	2	0	
Site of intestinal amebiasis			
Cecum	5		
Ascending	3		
Transverse	1		
Descending	0		
Sigmoid	1		
Rectum	4		

TABLE 3

Frequencies of HLA class II alleles in patients with and without amebiasis

	Patients with amebiasis ($N = 7$)	Patients without amebiasis ($N = 50$)	P value
DRB1			
*04:03	1 (14.3%)	5 (10.0%)	0.56
*04:05	3 (42.9%)	16 (32.0%)	0.68
*04:06	1 (14.3%)	5 (10.0%)	0.56
*09:01	1 (14.3%)	17 (34.0%)	0.41
*11:01	0 (0.0%)	6 (12.0%)	1.00
*13:02	0 (0.0%)	7 (14.0%)	0.58
*15:01	1 (14.3%)	7 (14.0%)	1.00
*15:02	3 (42.9%)	5 (10.0%)	0.050
DQB1			
*03:01	1 (14.3%)	11 (22.0%)	1.00
*03:02	2 (28.6%)	12 (24.0%)	1.00
*03:03	1 (14.3%)	20 (40.0%)	0.24
*04:01	3 (42.9%)	16 (32.0%)	0.68
*05:02	1 (14.3%)	3 (6.0%)	0.42
*05:03	0 (0.0%)	6 (12.0%)	1.00
*06:01	5 (71.4%)	5 (10.0%)	0.001
*06:02	1 (14.3%)	7 (14.0%)	1.00
*06:04	0 (0.0%)	7 (14.0%)	0.58

Data are numbers and frequencies of patients harboring each HLA allele. HLA data were available in 57 patients. HLA alleles identified in more than five patients (> 10%) were considered.

Additional studies are needed to examine the effects of host genetic factors on *E. histolytica* infection and the development of invasive disease. Interestingly, not only HLA but also, mutation of the leptin receptor were reported to be associated with amebic infection.[13]

In conclusion, intestinal amebic ulcerative lesions were frequently found in asymptomatic HIV-1–infected Japanese individuals who could otherwise act as reservoirs for new infection in other high-risk populations. Additional studies of subclinical infection are needed to control the *E. histolytica* endemicity.

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Acute Hepatitis C in HIV-1 Infected Japanese Cohort: Single Center Retrospective Cohort Study

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Abstract

Objectives: HCV co-infection is a poor prognostic factor in HIV-1-infected patients. Although the number of newly reported patients who show seroconversion is increasing, the clinical features are still unclear, especially in Asian countries.

Design: A single-center retrospective cohort study of patients diagnosed between 2001–2012.

Methods: Acute hepatitis C (AHC) was diagnosed upon detection of high serum ALT (>100 IU) followed by anti-HCV seroconversion. Clinical characteristics, HIV-1-related immunological status and IL-28B genotypes (rs12979860, rs8099917) were collected. We compared these variables between patients with and without spontaneous clearance of HCV and between responders and non-responders to treatment with pegylated interferon (PEG-IFN) plus ribavirin.

Results: Thirty-five patients were diagnosed with AHC during the study period. The majority (96.9%) were MSM. Three were lost to follow-up. Seventy-five percent of patients with AHC (24/32) were asymptomatic and found incidentally to have high serum ALT. Compared to those who did not show spontaneous clearance, patients with spontaneous HCV viral clearance showed more symptoms and more severe abnormalities related to acute hepatitis. Spontaneous clearance was seen in 4 out of 28 patients with CC+TT genotype, but not in 6 patients with IL-28B CT+TG genotype. PEG-IFN plus ribavirin treatment was initiated in 12 out of 28 cases without spontaneous clearance. The sustained virological response rate was high (81.8%, 9/11), even in cases with CT+TG genotype infected with HCV genotype 1b (SVR 2/2).

Conclusions: Careful attention to AHC is needed in HIV-1-infected MSM. Early diagnosis and PEG-IFN plus ribavirin treatment should be considered for AHC cases.

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All data supporting our conclusions are included within the manuscript. Original data of our retrospective analyses are available in medical records of our hospital.

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Introduction

The estimated worldwide prevalence of hepatitis C virus (HCV) infection is 2–3% [1]. HCV co-infection increases morbidity rate in HIV infected individuals, and previous meta-analysis reported mortality among patients co-infected with HCV was 1.35 times higher than that among patients with HIV-infection alone even in the highly active antiretroviral therapy (HAART) era [2]. In HIV-1/HCV co-infected patients, progression to liver cirrhosis and hepatocellular carcinoma (HCC) is faster than that in patients without HIV-1 infection [3]. Furthermore, the response to treatment with pegylated interferon (PEG-IFN) plus ribavirin (RBV) in HIV-positive patients with chronic HCV infection is poor (sustained virological response: SVR 19–40%), compared with patients infected with HCV alone (SVR 54–61%) [4–9].

The risk of HCV acquisition via heterosexual intercourse is estimated to be very low [10]. Recently, however, a high incidence of HCV seroconversion has been reported in HIV-1 infected men who have sex with men (MSM) [11–13]. These results suggest that new HCV infection can be a potential future problem in the clinical management of HIV-1 infected patients. On the other hand, a favorable response to treatment with PEG-IFN plus RBV for acute hepatitis C (AHC) relative to that for chronic one has been reported in HCV-infected (SVR 85–98%) [14,15] and HIV/HCV co-infected patients (SVR 60–80%) [16,17]. In this regard, the recent guidelines recommend PEG-IFN plus RBV treatment

for AHC in HIV-1 co-infected patients [18–20]. However, data of AHC among HIV-1 infected patients is still limited, especially from Asian countries.

The response to treatment with PEG-IFN plus RBV is closely associated with the interleukin-28B (IL-28B) genotype, which encodes interferon-λ3 (IFN-λ3), in chronic HCV hepatitis, even in HIV-1 co-infected cases [21–23]. Furthermore, HCV mono-infected individuals with favorable IL-28B genotype (CC at rs12979860, TT at rs8099917) seem to achieve spontaneous clearance of HCV compared to those with non-favorable genotypes [21,22,24,25]. To our knowledge, there are no studies on the effect of IL-28B genotype on the natural course and response to treatment of AHC in HIV-1 infected individuals in Asian population [24,26,27].

In the last 12 years, 35 patients with HIV-1 infection were diagnosed with AHC in our hospital. In the present retrospective study, we report the results of analysis of data of 32 of these cases, and discuss the factors associated with spontaneous HCV clearance and response to treatment with PEG-IFN plus RBV (Fig. 1).

Methods

Study Design

This single-center retrospective cohort study was conducted in accordance with the ethical principles of the Declaration of Helsinki and of Good Clinical Practice. The ethics committee of National Center for Global Health and Medicine approved the study. All patients provided written informed consent.

Study Participants

The medical records of HIV-1 infected patients in our institution, the largest HIV clinic in Japan, admitted and treated between January 2001 and December 2012, were retrospectively reviewed. AHC was defined according to the following criteria; elevation of alanine transferase (ALT) >100 IU/L accompanied by seroconversion of anti-HCV antibody, and exclusion of other causes (e.g., acute hepatitis B and drug-induced hepatitis). Patients who were lost to follow-up within 1 year from the diagnosis of AHC were excluded from the analysis since they could not be assessed for clinical presentation including spontaneous clearance. Spontaneous clearance was defined as a decrease in HCV RNA to undetectable level without treatment within one year from the diagnosis and remaining as such thereafter. For patients receiving PEG-IFN plus RBV treatment, we assessed the SVR rate. SVR was defined as continued undetectable HCV RNA at 24 weeks

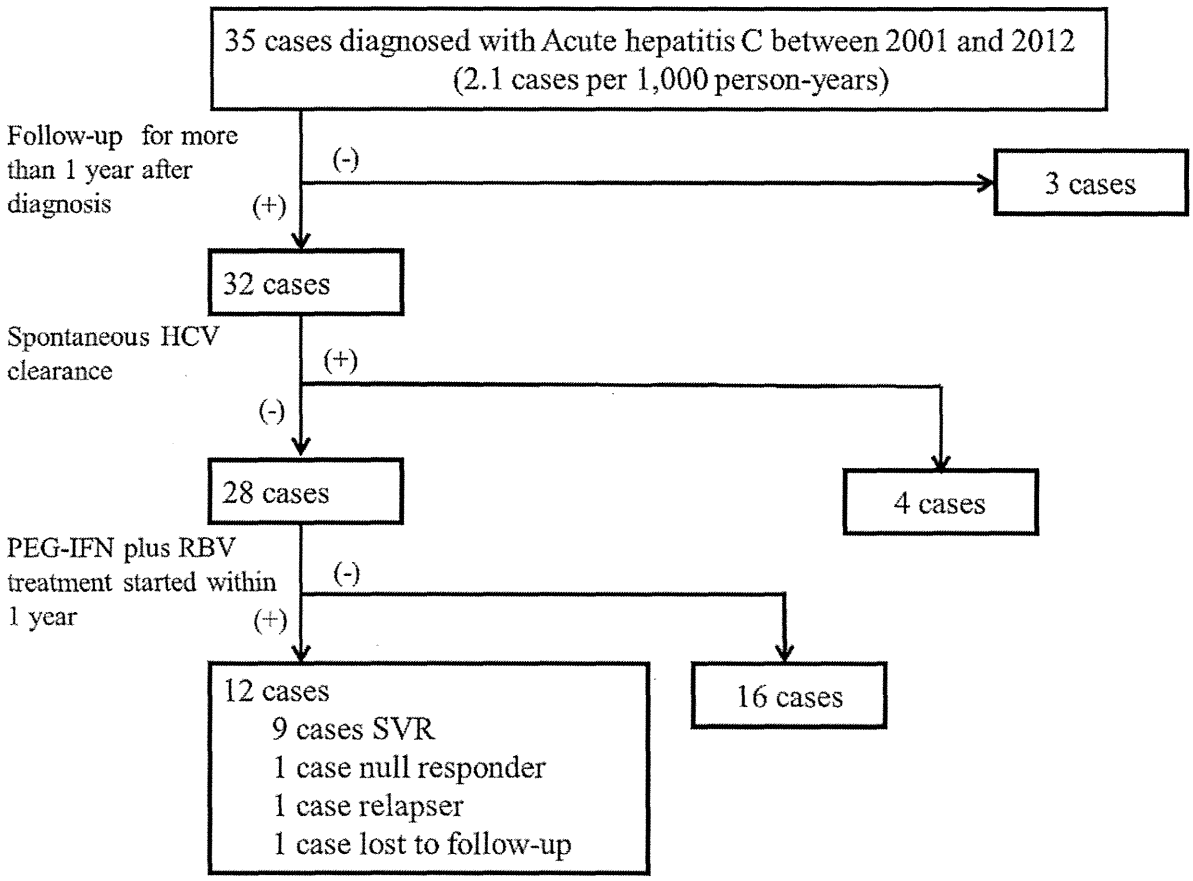

Figure 1. Patient enrollment process. Acute hepatitis C (AHC) was defined as elevation of alanine transaminase (ALT) >100 IU/L accompanied by seroconversion of anti-hepatitis C virus (HCV) antibody. Three patients could not be followed up for 1 year after diagnosis and were excluded from further analysis. HCV cleared spontaneously in 4 cases. PEG-IFN plus RBV treatment was initiated within 1 year of diagnosis of AHC in 12 out of 28 patients who did not show spontaneous clearance. One patient with missing treatment data following transfer to another clinic about two weeks after initiation of IFN plus RBV, was excluded from analysis related to the effect of PEG-IFN plus RBV. PEG-IFN: pegylated interferon, RBV: ribavirin.
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Table 1. Characteristics of AHC patients (n = 32).

	All patients (n = 32)	Spontaneous clearance (n = 4)	Non-spontaneous clearance (n = 28)	P-value
Age (years)	40 [30–58]	44 [37–56]	40 [30–58]	0.361
Male sex	32 (100)	4 (100)	28 (100)	-
Men who have sex with men	31(96.9)	4 (100)	27 (96.4)	1.000
IL-28B genotypes (rs12979860+rs8099917)				
CC+TT genotype	26 (81.2)	4 (100)	22 (78.6)	0.416
CT+TG genotype	6 (18.8)	none	6 (21.4)	-
TT+GG genotype	none	none	none	-
Injecting drug users	4 (12.5)	none	4 (14.3)	1.000
Received ART at diagnosis	29 (90.6)	4 (100)	25 (89.3)	1.000
CD4 count at diagnosis (cells/μL)	420 [167–824]	317 [184–616]	424 [167–824]	0.424
HIV-RNA at diagnosis (copies/mL)	UD [UD−9.4×10^4]	50 [UD-50]	42.5 [UD-9.4×10^4]	0.737

Data are number (%) of patients or median [range].
ART, antiretroviral therapy; UD, undetectable.
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after completion of therapy. Baseline characteristics, status of HIV-1 infection, history of injecting drug usage (IDU), symptoms related to AHC (fatigue and jaundice), laboratory data abnormalities from AHC (ALT, T-bil), treatment of HCV infection and histological findings of liver biopsy, where available, and were collected from the medical records. We compared these variables between patients with and without spontaneous clearance of HCV and between responders and non-responders to treatment with PEG-IFN plus RBV.

HCV Analysis

For each patient, titers of anti-HCV antibody were measured by a third-generation Latex aggregation assay (Ortho HCV Ab LPIA test III, Ortho Clinical Diagnostics, NJ) at first visit to our hospital and at diagnosis of AHC. Serum HCV RNA at each time point was extracted automatically (Cobas Ampliprep, Roche InVitro Diagnostics, Switzerland). Thereafter, cDNA was prepared and its titer was measured by quantitative polymerase chain reaction (Cobas TaqMan 48, Roche In Vitro Diagnostics). Direct sequencing was performed using DNA probe assay by ABI PRISM 3100 (Applied Biosystems, Foster City, CA). Finally, the

Table 2. Clinical presentation of AHC patients (n = 32).

	All patients (n = 32)	Spontaneous clearance (n = 4)	Non-spontaneous clearance (n = 28)	P-value
No symptoms	24 (75)	1 (25)	23 (82.1)	-
Symptoms	8 (25)	3 (75)	5 (17.9)	0.039
Fatigue	8 (25)	3 (75)	5 (17.9)	-
Jaundice	2 (6.25)	1 (25)	1(3.6)	-
Peak Alanine transaminase level (IU/L)	661 [117–2194]	707 [1237–2126]	614 [117–2194]	0.072
Peak total bilirubin level (mg/dL)	1.9 [0.7–17.0]	9.8 [4.2–17.0]	1.6 [0.7–6.8]	0.002
HCV genotype				
1a	1/27 (3.7)	None	1/2 (4.3)	-
1b	19/27 (70.4)	3/4 (75)	16/23 (69.6)	-
2a	4/27 (14.8)	1/4 (25)	3/23 (13)	-
2b	3/27 (11.1)	None	3/23 (13)	-
Not available	5	None	5	
HCV-RNA at diagnosis (Log IU/mL)	6.6 [1.9–7.8]¶	6.6 [4.9–6.8]†	6.6 [1.9–7.8]‡	0.594
Latency to HCV clearance (wks)*	-	11 [7–31]	-	-

Data are number (%) of patients or median [range] values.
*Time between AHC diagnosis and HCV clearance (weeks).
¶Data of 6 patients not available for analysis.
†Data of 5 patients not available for analysis.
‡Data of 1 patient not available for analysis.
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Table 3. Comparison of patients of the SVR and non-SVR groups.

	All patients (n = 11)	SVR (n = 9)	Non-SVR (n = 2)
Age (years)	38 [30–58]	38 [30–48]	52 [47–58]
Male sex	11 (100)	9 (100)	2 (100)
Men who have sex with men	11 (100)	9 (100)	2 (100)
IL-28B genotype			
CC+TT genotype	9 (81.8)	7 (77.8)	2 (100)
CT+TG genotype	2 (18.2)	2 (22.2)	None
Injecting drug users	1 (9.1)	None	1 (50)
Received ART before treatment	10 (90.9)	8 (88.9)	2 (100)
CD4 count before treatment (cells/μL)	382 [230–655]	440 [272–655]	238 [254–278]
HIV-RNA before treatment (copies/mL)	UD [UD-3.3×10^4]	UD [UD-3.3×10^4]	UD [305–610]
HCV genotype			
1b	10 (90.9)	8 (88.9)	2 (100)
2a	1 (9.1)	1 (11.1)	None
HCV-RNA before treatment (Log IU/mL)	6.3 [3.3–7.8]	6.3 [5–7.8]	5.7 [3.6–8.0]
Latency to AHC diagnosis (months)*	3.2 [0.9–6.9]	3.2 [0.9–6.9]	4.4 [3.7–5.1]
Duration of PEG-IFN+RBV therapy (wks)	43 [11–72]	43 [11–72]	36 [11–60]
Latency to HCV clearance (wks)¶	-	8 [3–16]	-
RVR	3 (27.2)	3 (33.3)	None
EVR	7 (63.6)	6 (66.7)	1 (50)
Histopathology positive for liver fibrosis			
F0	3/6	3	0
F1	2/6	1	1
F2	1/6	0	1
F3	0	0	0

Data are number (%) of patients or median [range] values.
*Time between AHC diagnosis and initiation of therapy (months).
¶Time between initiation of therapy and HCV clearance (weeks).
ART, antiretroviral therapy; UD, undetectable; PEG-IFN+RBV, pegylated interferon plus ribavirin; SVR, sustained viral response; EVR, early viral response; RVR, rapid viral response.
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genotype was determined from the amino acid sequences of 5 – untranslated region [28].

Genotyping of IL-28b Alleles

Genomic DNA was isolated from peripheral blood mononuclear cells, using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). SNPs, rs12979860, and rs 8099917 were genotyped, using the TaqMan Drug Metabolism Assays by ABI PRISM 7900 HT sequence detection system (Applied Biosystems) according to the instructions provided by the manufacturer. The researchers responsible for genotyping were blinded to clinical data of the patients.

Statistical Analysis

The patients' characteristics and results of differences in viral clearance and virological response were compared using chi-square test (for qualitative variables) or Mann-Whitney U-test (for quantitative variables). Statistical significance was defined at two-sided p value of <0.05. All statistical analyses were performed with The Statistical Package for Social Sciences Version 21 (SPSS Inc, Chicago, IL).

Results

Patient Enrollment

A total of 35 patients were diagnosed with AHC during the study period. The incidence of AHC was 2.1 cases per 1,000 person-years. Three patients who were lost to follow-up within 1 year after diagnosis of AHC were excluded from the analysis. No deaths or fulminant hepatitis were recorded during the study period. Spontaneous HCV clearance was achieved by 4 patients, including 2 patients in whom HCV clearance was achieved within 3 months of diagnosis of AHC. The median time between diagnosis of AHC and HCV clearance was 11 weeks (range, 7–31 weeks). Among the 28 patients who did not show spontaneous HCV clearance, treatment with PEG-IFN plus RBV was initiated within 6 months of diagnosis in 9 patients and between 6 and 12 months of diagnosis of AHC in 3 patients (6.1, 6.4 and 6.9 months, respectively), whereas treatment was not initiated in the remaining 16 patients due to cost (n = 7) or other comorbidity (depression, history of epilepsy) (**Fig. 1**).

Patients' Characteristics and Clinical Presentations of AHC

The characteristics and clinical presentation of AHC patients are listed in **Tables 1** and **2**, respectively. All patients were

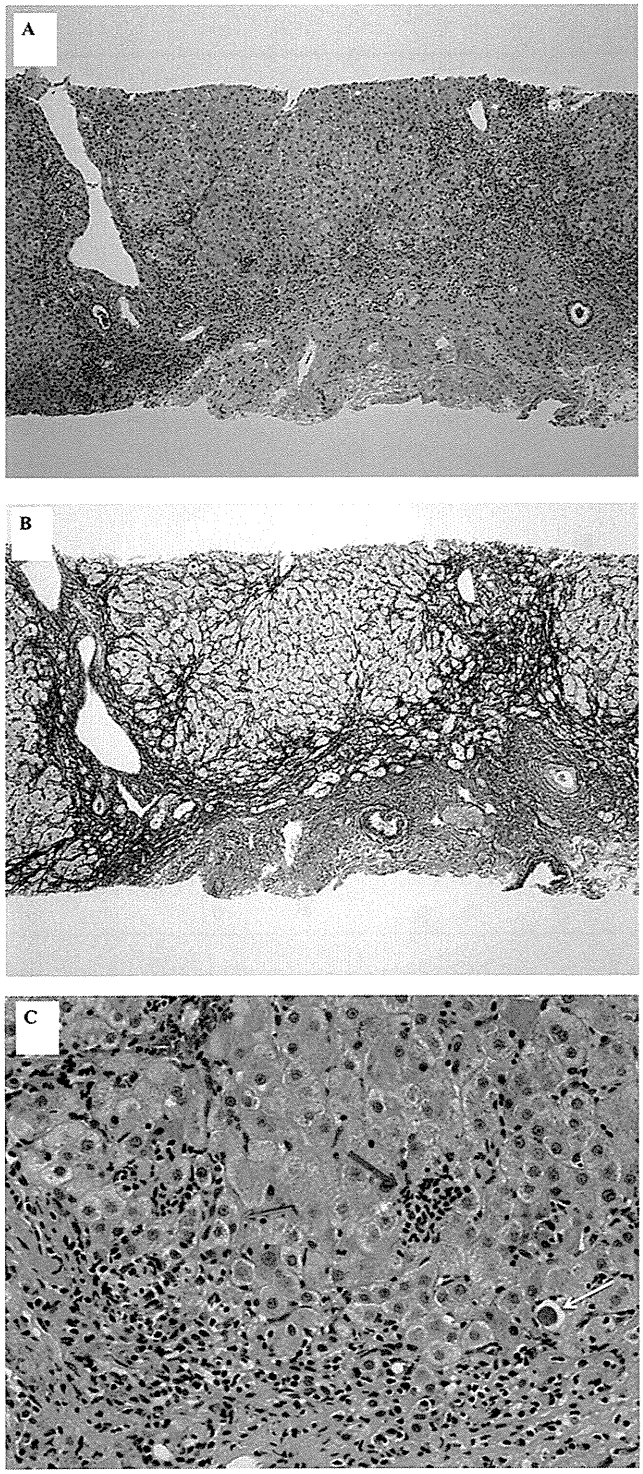

Figure 2. Histological findings in needle liver biopsy specimen from the patient who showed null-response (Table 3). The pre-treatment biopsy specimen obtained at 13 weeks after AHC diagnosis showed stage 2 fibrosis (F2) according to the classification of chronic hepatitis C (New Inuyama Classification). (A and B) Formation of bridging fibrosis by fibrous and cellular expansion in the portal tract. (C) Magnified view showing centrilobular piece-meal necrosis (green arrow), acid folic body (yellow arrow) and spotty necrosis (red arrow). (A) Hematoxylin-eosin stain, x100, (B) Silver impregnation stain, x100, (C) Hematoxylin-eosin stain, x400. PEG-IFN: pegylated interferon, RBV: ribavirin, AHC: acute C hepatitis.
doi:10.1371/journal.pone.0100517.g002

Japanese men, including 31 (96.9%) MSM. Twenty nine patients (90.6%) received antiretroviral therapy (ART) and HIV-RNA was well suppressed in these patients. Four patients (12.5%) had a history of IDU, whereas none had a history of occupational exposure to HCV or blood transfusion. Although there was no significant difference between patients with and without spontaneous HCV clearance, the IL-28B CC+TT genotype was rs12979860 and rs8099917 in all 4 patients who showed spontaneous clearance and none of the patients with IL-28B CT+TG genotype showed spontaneous clearance (**Table 1**).

The majority of patients with AHC (24/32, 75%) were asymptomatic at the onset of AHC. High ALT was identified incidentally at routine visit for HIV-1 infection, with subsequent tests confirming the diagnosis of AHC. Compared to patients who did not show spontaneous clearance, patients with spontaneous clearance showed more severe clinical presentation of hepatitis (symptomatic, with higher serum total bilirubin and ALT value at diagnosis). The most frequent HCV genotype was 1b (70.4%). At diagnosis, HCV RNA was higher than 5.0 LC/mL in 24 out of 26 patients (**Table 2**).

Response to Treatment with PEG-IFN Plus RBV

Treatment with PEG-IFN plus RBV was initiated in 12 patients within 1 year of diagnosis of AHC (median interval from AHC diagnosis, 3.2 months). We assessed the response to treatment in only 11 patients; the other patient was lost to follow-up within two weeks of treatment initiation (**Fig. 1**, **Table 3**). SVR was achieved in 9 of 11 patients (81.8%) despite the high incidence of HCV genotype 1b and high viral load.

Two patients did not achieve SVR. Both patients were infected by genotype 1b with high viral load, and treatment was initiated within 6 months of diagnosis. One achieved viral clearance within 12 weeks (early virological response: EVR) but showed viral rebound at 15 weeks after completion of the treatment (relapser), whereas viral clearance was not achieved during treatment in the other patient (null-responder). Both patients were relatively older and their CD4 counts were lower, compared to those with SVR, although statistical analysis was not performed due to the small number of cases. In patients with SVR, the median time between initiation of therapy and clearance of HCV was 8 weeks (range, 3–16 weeks). Surprisingly, both patients with IL-28B CT+TG alleles achieved SVR despite genotype 1b and high viral load, although we could not compare the SVR rate among different genotypes since only one patient was infected with genotype 2a in this study.

Histological Findings of AHC in HIV-1 Co-infected Patients

HBs antigen was negative and ALT was within the normal range in the year preceding AHC in all 6 patients, whereas HBs Ab and/or HBc Ab was positive in 5 patients. No pre-existing factors of liver fibrosis other than HIV infection were evident before AHC. Liver biopsy was performed in 6 patients before treatment with PEG-IFN plus RBV. The median interval between diagnosis of AHC and biopsy was 4.3 months (range, 3.3–6.1 months). Fibrotic changes were confirmed in 3 cases by hematoxylin-eosin staining and silver impregnation staining (**Fig. 2**, **Table 3**). These lesions were paler-staining by Victoria Blue stain, indicating that the fibrotic areas did not reflect chronic changes.

Discussion

In the present study, we identified 35 cases of AHC during the study period and nearly all such patients (34/35) were MSM, and

the most frequent HCV genotype was 1b (19/27). These findings are consistent with previous reports from other countries [11–13]. In this regard, a high incidence of HCV seroconversion in HIV-1 infected MSM was reported recently by two separate groups [11–13]. The same studies also reported that genotype 1b was the major genotype among their patients [11–13], and that HCV infection was frequently not detected during the acute phase and diagnosed only at the chronic stage mainly due to the lack of symptoms.

Similar to the previous reports on AHC, 75% of our cases were asymptomatic, and only 6.3% of the study population showed mild elevation of serum ALT (100 IU/L < ALT <150 IU/L). In this regard, ALT elevation during acute HCV infection is often relatively transient, and therefore could be easily missed during routine clinical care. The need of regular screening for anti-HCV antibody in HIV-1 infected MSM is controversial, and the recommendations are different in guidelines from different developed countries [29,30]. Our results emphasize the importance of regular ALT monitoring and HCV re-screening at the time of mild ALT elevation during follow-up, especially in high-risk populations such as sexually active MSM.

There are few reports on the relationship between IL-28B CC+TT genotype and spontaneous clearance of HCV [21,31]. In the present study, spontaneous HCV clearance was seen in 4 out of 26 patients with IL-28B CC+TT genotype, whereas no spontaneous HCV clearance was seen in all 6 patients with IL-28B CT+TG genotype. Although this difference could not be confirmed to be statistically significant due to the small number of patients (4 patients), this is, to our knowledge, the first report on the relation between IL-28B and spontaneous HCV clearance during AHC in HIV-1 co-infected patients in Asian population. Our study also showed that the severity of clinical symptoms was an important factor related to spontaneous HCV clearance. Further investigation is needed for a better understanding of the pathogenesis of AHC, especially factors involved in spontaneous clearance.

The use of PEG-IFN plus RBV treatment for AHC within 6 months of diagnosis is now recommended for HIV-1 co-infected cases [17–19] although data on the response of HIV-1 infected individuals with AHC to the PEG-IFN plus RBV remain limited. One study reported spontaneous clearance of HCV between 6 and 12 months of diagnosis [32]. In this regard, it is sometimes difficult in the clinical setting to start PEG-IFN plus RBV treatment within 6 months of diagnosis because some patients have comorbidities and complications other than HIV and HCV. In our analysis, 9 of 11 patients (81.8%), including 2 patients whose treatment was initiated between 6 and 12 months of diagnosis, achieved SVR despite high rate of genotype 1b infection (SVR 90.0% among those with genotype 1b virus). Furthermore, HCV genotype 1b-infected patients carrying the IL-28B CT+TG genotype (n = 2), which is a predictor of poor response to the treatment of chronic HCV infection, achieved SVR. These results emphasize the advantage of the PEG-IFN plus RBV treatment for AHC.

Little is known about the progression of AHC to liver fibrosis in patients with HIV/HCV co-infection [33], although rapid progression of liver fibrosis during the chronic phase is well recognized [3]. Fierer et al. [34] reported that the development of fibrosis occurs even in the acute phase of HCV infection in HIV-Infected men. In the present study with limited cross-sectional analysis of liver biopsies after AHC, fibrosis was detected in 3 out of 6 cases, which is consistent with the above report of Fierer et al. [34]. Moreover, SVR was not achieved in 2 out of 3 patients who showed liver fibrosis, whereas the other 3 patients without fibrosis achieved SVR (**Table 3**). These results emphasize the clinical importance of early diagnosis and early treatment for AHC in HIV-1 infected individuals.

In conclusion, the potential of AHC should always be considered in HIV-1 infected MSM, even in asymptomatic case, who present with mild ALT elevation. Favorable response can be expected if anti-HCV treatment is initiated during the early phase. Further investigation is needed to determine the predictor(s) of spontaneous HCV clearance, appropriate timing of treatment initiation, and duration of treatment.

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Author Contributions

Conceived and designed the experiments: MI KW TK YK SO HG. Performed the experiments: MI KW TK YN MY TI NM. Analyzed the data: MI KW TK HG. Contributed reagents/materials/analysis tools: MI KW TK YN MY TI NM YK SO HG. Contributed to the writing of the manuscript: MI KW KT HG.

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