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.

Acknowledgments

The authors thank Hirokazu Kumagai, deputy chief radiology technologist, Mikiko Ogata, Michiyo Ishisaka, and

Misao Takano for their invaluable contribution to the study. The authors also thank Akiko Nakano, the study coordinator, and all the other staff at the AIDS Clinical Center for their help in the completion of this study.

This work was supported by a Grant-in Aid for AIDS research from the Japanese Ministry of Health, Labour, and Welfare (H23-AIDS-001; H24-AIDS-003).

Author Disclosure Statement

No competing financial interests exist.

References

1. Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents: Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed February 27, 2014.
2. Garvey L, Winston A, Walsh J, *et al.:* HIV-associated central nervous system diseases in the recent combination antiretroviral therapy era. Eur J Neurol 2011;18:527–534.
3. d'Arminio Monforte A, Cinque P, Mocroft A, *et al.:* Changing incidence of central nervous system diseases in the EuroSIDA cohort. Ann Neurol 2004;55:320–328.
4. Mocroft AJ, Lundgren JD, d'Armino Monforte A, *et al.:* Survival of AIDS patients according to type of AIDS-defining event. The AIDS in Europe Study Group. Int J Epidemiol 1997;26:400–407.
5. Tan IL, Smith BR, von Geldern G, *et al.:* HIV-associated opportunistic infections of the CNS. Lancet Neurol 2012;11:605–617.
6. Antinori A, Larussa D, Cingolani A, *et al.:* Prevalence, associated factors, and prognostic determinants of AIDS-related toxoplasmic encephalitis in the era of advanced highly active antiretroviral therapy. Clin Infect Dis 2004;39:1681–1691.
7. Cinque P, Koralnik IJ, Gerevini S, *et al.:* Progressive multifocal leukoencephalopathy in HIV-1 infection. Lancet Infect Dis 2009;9:625–636.
8. Clifford DB and Ances BM: HIV-associated neurocognitive disorder. Lancet Infect Dis 2013;13:976–986.
9. Utsuki S, Oka H, Abe K, *et al.:* Primary central nervous system lymphoma in acquired immune deficiency syndrome mimicking toxoplasmosis. Brain Tumor Pathol 2011;28:83–87.
10. Graham CB 3rd, Wippold FJ 2nd, Pilgram TK, *et al.:* Screening CT of the brain determined by CD4 count in HIV-positive patients presenting with headache. AJNR Am J Neuroradiol 2000;21:451–454.
11. Nishijima T, Kawasaki Y, Tanaka N, *et al.:* Long-term exposure to tenofovir continuously decrease renal function in HIV-1-infected patients with low body weight. AIDS 2014:1.
12. Zolopa A, Andersen J, Powderly W, *et al.:* Early antiretroviral therapy reduces AIDS progression/death in individuals with acute opportunistic infections: A multicenter randomized strategy trial. PLoS One 2009;4:e5575.
13. McLeod DS, Mortimer RH, Perry-Keene DA, *et al.:* Histoplasmosis in Australia: Report of 16 cases and literature review. Medicine (Baltimore) 2011;90:61–68.
14. Ustianowski AP, Sieu TP, and Day JN: Penicillium marneffei infection in HIV. Curr Opin Infect Dis 2008;21:31–36.
15. Antinori A, Arendt G, Becker JT, *et al.:* Updated research nosology for HIV-associated neurocognitive disorders. Neurology 2007;69:1789–1799.

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Katano et al. BMC Infectious Diseases 2014, 14:229
http://www.biomedcentral.com/1471-2334/14/229

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

The prevalence of opportunistic infections and malignancies in autopsied patients with human immunodeficiency virus infection in Japan

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Abstract

Background: Opportunistic infections and malignancies such as malignant lymphoma and Kaposi sarcoma are significant complications of human immunodeficiency virus (HIV) infection. However, following the introduction of antiretroviral therapy in Japan in 1997, the incidence of clinical complications has decreased. In the present study, autopsy cases of HIV infection in Japan were retrospectively investigated to reveal the prevalence of opportunistic infections and malignancies.

Methods: A total of 225 autopsy cases of HIV infection identified at 4 Japanese hospitals from 1985–2012 were retrospectively reviewed. Clinical data were collected from patient medical records.

Results: Mean CD4 counts of patients were 77.0 cells/μL in patients who received any antiretroviral therapy during their lives (ART (+) patients) and 39.6 cells/μL in naïve patients (ART (–) patients). Cytomegalovirus infection (142 cases, 63.1%) and *pneumocystis* pneumonia (66 cases, 29.3%) were the most frequent opportunistic infections, and their prevalence was significantly lower in ART (+) patients than ART (–) patients. Non-Hodgkin lymphoma and Kaposi sarcoma were observed in 30.1% and 16.2% of ART (–) patients, and 37.9% and 15.2% of ART (+) patients, respectively. Malignant lymphoma was the most frequent cause of death, followed by cytomegalovirus infection regardless of ART. Non-acquired immunodeficiency syndrome (AIDS)-defining cancers such as liver and lung cancer caused death more frequently in ART (+) patients (9.1%) than in ART (–) patients (1.5%; $P = 0.026$).

Conclusions: The prevalence of infectious diseases and malignancies were revealed in autopsy cases of HIV infection in Japan. The prevalence of cytomegalovirus infection and *pneumocystis* pneumonia at autopsy were lower in ART (+) patients than ART (–) patients. Higher prevalence of non-AIDS defining malignancies among ART (+) patients than ART (–) patients suggests that onsets of various opportunistic infections and malignancies should be carefully monitored regardless of whether the patient is receiving ART.

Keywords: AIDS, Opportunistic infections, Autopsy, Antiretroviral therapy

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Background

Opportunistic infections such as *Pneumocystis jirovecii* pneumonia (PCP), cytomegalovirus (CMV), non-tuber culous mycobacteria (NTM), and fungal infections are frequently found in patients with acquired immunodeficiency syndrome (AIDS) [1]. The most frequent opportunistic infection among patients with AIDS is CMV infection, which commonly causes retinitis, pneumonia, and gastrointestinal tract ulcers. PCP is also a frequent infectious disease in the lungs of patients with AIDS. Additionally, malignancies such as non-Hodgkin lymphoma (NHL) and Kaposi sarcoma (KS) are significant complications. NHL in particular is not easily controlled and is a frequent AIDS-associated cause of death. Interestingly, KS has only been reported in homosexual patients, and patients with multifocal KS lesions have a poor prognosis.

The introduction of antiretroviral therapy (ART) has drastically changed the incidence of opportunistic infections in patients infected with human immunodeficiency virus 1 (HIV-1), resulting in a decline in mortality rates [2-7]. ART has decreased the frequencies of CMV, PCP, and NTM infections in patients with AIDS [7]; however, the frequency of NHL has not changed dramatically [8]. Additionally, non-AIDS-defining malignancies such as liver, lung, and gastric cancers have been observed in patients with AIDS, regardless of ART [9]. A recent study demonstrated that low CD4 counts at ART initiation was associated with a greater risk of KS and lymphoma, whereas other cancers increased over time with ART, likely reflecting an increased risk of cancer with aging [10], low CD4 counts, and cigarette smoking [11-13].

Although mortality rates have decreased dramatically with the use of ART, its effect in many patients with AIDS is limited, and AIDS-associated complications remain a leading cause of death [14,15]. Additionally, untreated HIV-1-positive patients with severe AIDS-defining illnesses frequently visit hospitals and often rapidly succumb to suddenly aggressive progression of their illness [16,17]. Systematic pathological analysis of autopsy cases can provide useful information related to the cause of death and the distribution of pathogens in patients. However, there have been few reports describing the prevalence of infectious diseases and malignancies in autopsied patients with HIV infection [1,18]. A previous study using samples from autopsied patients with HIV infection during 1982–1998 demonstrated the prevalence of CMV, PCP, and NTM infections decreased during the study period [18]. The same study reported that, although the prevalence of KS was unchanged, the prevalence of NHL increased during the study period [18]. To the best of our knowledge, there are no reports demonstrating changes in the prevalence of opportunistic infections in autopsy cases of HIV infection following the introduction of ART after 2000.

In the present study, autopsy cases of HIV infection in Japan were retrospectively investigated to determine the prevalence of opportunistic infections and malignancies often found in patients with AIDS, including non-AIDS-defining malignancies. Additionally, the association of ART use with the prevalence of opportunistic infections and malignancies was investigated.

Patients and methods

Patients

The present study was approved by the Institutional Review Board of the National Institute of Infectious Diseases (Approval No. 356) and of four hospitals in Japan: Tokyo Metropolitan Komagome Hospital, National Center for Global Health and Medicine, Research Hospital, the Institute of Medical Science, the University of Tokyo, and Osaka National Hospital. Each hospital enrolled in the present study is a central hospital for AIDS treatment in Tokyo and Osaka, and has performed more than 15 autopsies of patients infected with HIV. According to a national autopsy survey by the Japan Pathology Society, 828 patients infected with HIV were autopsied in Japan from 1987–2009. During the period 1985–2009, 215 patients infected with HIV were autopsied at the 4 aforementioned hospitals. Thus, the number of cases in this study covered approximately 26% of all autopsied HIV cases. Ten cases autopsied in the period 2010–2012 were added to the 215 cases, making a total of 225 patients analyzed in this study (Table 1), of which 95.1% were male. The patients' ages at death ranged from 12 to 80 years, with a mean age of 44.4 years (median 44 years). Among them, 35.6% were homosexual, and 29.3% received ART (Table 1). The mean CD4 count at the last blood examination before death was 51.5 cells/μL (range: 0–560 cells/μL; median: 13.5 cells/μL). ART was introduced in Japan in 1997. In this study, ART was defined as any combination of therapy that included two nucleoside or nucleotide reverse transcriptase inhibitors plus a non-nucleoside reverse transcriptase inhibitor, protease inhibitor, or abacavir (another nucleotide reverse transcriptase inhibitor). Additionally, ART (+) patients were defined as patients who received any ART during their lifetime, whereas ART (−) patients were as patients who did not receive ART.

Methods

Pathological findings were collected from autopsy records. CMV infection was determined by the infiltration of large cells with typical inclusion bodies. Infections by other viral agents such as hepatitis B virus, herpes simplex virus, hepatitis C virus, JC virus (causing progressive multifocal leukoencephalopathy), and varicella zoster virus were confirmed by immunohistochemistry or polymerase chain reaction. HIV encephalopathy was defined by morphological features indicating the presence of syncytial

Table 1 Characteristics of the patients infected with HIV

Factors	Groupings	Total patients		ART (–) patients		ART (+) patients		*P* value
		n	%	n	%	n	%	
Total		225*	100%	136	100%	66	100%	
Sex	Male	214	95.1%	128	94.1%	63	95.5%	0.695**
	Female	11	4.9%	8	5.9%	3	4.5%	
Age at death	<10 years	0	0.0%	0	0.0%	0	0.0%	**0.028****
	11–20	2	0.9%	2	1.5%	0	0.0%	
	21–30	30	13.3%	22	16.2%	6	9.1%	
	31–40	60	26.7%	34	25.0%	19	28.8%	
	41–50	69	30.7%	48	35.3%	14	21.2%	
	51–60	34	15.1%	18	13.2%	14	21.2%	
	61–70	24	10.7%	10	7.4%	10	15.2%	
	71–80	5	2.2%	1	0.7%	3	4.5%	
	>81	0	0.0%	0	0.0%	0	0.0%	
	Unknown	1	0.4%	1	0.7%	0	0.0%	
Risk factor	Homosexual	80	35.6%	52	38.2%	24	36.4%	0.800**
	Heterosexual	38	16.9%	24	17.6%	10	15.2%	
	Blood product	37	16.4%	29	21.3%	7	10.6%	
	Other	9	4.0%	5	3.7%	4	6.1%	
	Unknown	61	27.1%	26	19.1%	21	31.8%	
CD4 count before death	<50 cells/μL	122	54.2%	80	58.8%	33	50.0%	0.639***
	51–100	25	11.1%	11	8.1%	14	21.2%	
	101–200	13	5.8%	4	2.9%	9	13.6%	
	201–300	5	2.2%	5	3.7%	0	0.0%	
	301–400	3	1.3%	1	0.7%	2	3.0%	
	>401	4	1.8%	1	0.7%	3	4.5%	
	Unknown	53	23.6%	34	25.0%	5	7.6%	

ART, antiretroviral therapy. ART (+) patients were defined as patients who received any ART during their lifetime, whereas ART (–) patients were as patients who did not receive any ART in their lifetime.
*Total number of patients = 225 and included 23 patients with unknown ART status.
***P* values were calculated for the rates of male or homosexuals between ART (–) and (+) patients by Chi-square test.
****P* values were calculated for age or CD4 counts between all ART (–) and (+) patients by Mann–Whitney *U*-test. Bold font indicates statistical significance.

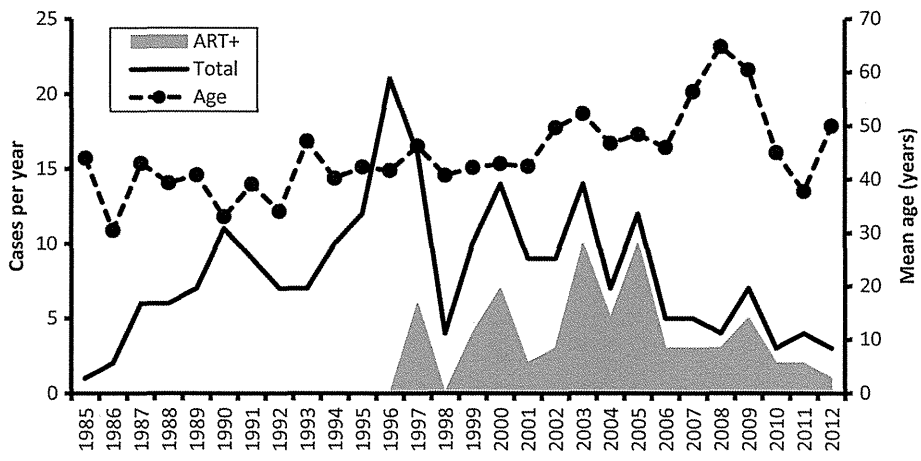

Figure 1 Annual number and mean age of AIDS-related autopsies. The solid line indicates total number of AIDS autopsies in each year. The gray area indicates the number of patients on ART in these autopsy cases. The broken bar indicates the mean age.

giant cells and detection of HIV-1 antigen by immunohistochemistry in the brain. Bacterial infection was identified by Gram stain, and in some cases, species of bacteria were identified by bacterial cultures. Tuberculosis and NTM infection were determined by acid-fast stain and/or PCR. Fungal and protozoan infections such as PCP, toxoplasma, *Candida*, *Aspergillus*, and *Cryptococcus* infection, were determined morphologically using Grocott's methenamine silver stain, periodic acid-Schiff stain, or/and immunohistochemistry. The histological sub-typing of malignant lymphoma was based on the World Health Organization classification, fourth edition. KS was confirmed by immunohistochemistry for Kaposi sarcoma-associated herpesvirus-encoded latency-associated nuclear antigen 1. Causes of death were determined by pathologists at each hospital based on the severity, distribution, and type of illness in the pathological findings of autopsy. Clinical data, such as age at autopsy, sex, risk factors, CD4 cell counts at the last blood examination before death, and use of ART in their lifetime were collected from medical records. Analysis of statistical significance was carried out using Mann–Whitney *U*-test for non-parametric two-sample analysis and Chi-squared test for contingency table analysis.

Results

After the introduction of ART in Japan in 1997, the total number of autopsies conducted on patients with HIV infection has slowly decreased whereas the mean age at autopsy has increased slightly (Figure 1). After 1997, 66

Table 2 Infectious diseases and malignancies in AIDS-associated autopsies

	All patients		ART (–) patients		ART (+) patients			
	n	%	n	%	n	%	*P* values	
Total	225	100.0%	136	100.0%	66	100.0%		
Infectious diseases								
Cytomegalovirus	142	63.1%	97	71.3%	25	37.9%	**<0.001**	
Pneumocystis jirovecii pneumonia	66	29.3%	43	31.6%	11	16.7%	**0.024**	
Non-tuberculous mycobacterium	31	13.8%	20	14.7%	8	12.1%	0.618	
Candida	25	11.1%	17	12.5%	6	9.1%	0.474	
Aspergillus	24	10.7%	17	12.5%	4	6.1%	0.160	
Human immunodeficiency virus encephalopathy	21	9.3%	13	9.6%	6	9.1%	0.915	
Cryptococcus	16	7.1%	11	8.1%	3	4.5%	0.526	Y
Hepatitis B virus	12	5.3%	6	4.4%	5	7.6%	0.549	Y
Herpes simplex virus	12	5.3%	1	0.7%	1	1.5%	0.816	Y
Toxoplasmosis	11	4.9%	9	6.6%	3	4.5%	0.789	Y
Hepatitis C virus	9	4.0%	3	2.2%	5	7.6%	0.147	Y
Progressive multifocal leukoencephalopathy	8	3.6%	4	2.9%	2	3.0%	0.684	Y
Tuberculosis	6	2.7%	4	2.9%	0	0.0%	0.385	Y
Varicella zoster virus	4	1.8%	2	1.5%	2	3.0%	0.835	Y
Multicentric Castleman disease	2	0.9%	1	0.7%	1	1.5%	0.816	Y
Malignancies								
Non Hodgkin lymphoma	71	31.6%	41	30.1%	25	37.9%	0.272	
Kaposi sarcoma	38	16.9%	22	16.2%	10	15.2%	0.852	
Endocervical cancer	0	0.0%	0	0.0%	0	0.0%	-	
Non-AIDS defining malignancies	20	8.9%	10	7.4%	10	15.2%	0.082	
Hepatic cancer	8	3.6%	4	2.9%	4	6.1%	0.495	Y
Lung cancer	6	2.7%	2	1.5%	4	6.1%	0.174	Y
Leukemia	2	0.9%	0	0.0%	2	3.0%	0.200	Y
Hodgkin lymphoma	2	0.9%	1	0.7%	1	1.5%	0.816	Y
Gastric cancer	1	0.4%	1	0.7%	0	0.0%	0.711	Y
Other cancer	3	1.3%	3	2.2%	0	0.0%	0.551	Y

P values were calculated by Chi-square test. Y indicates the use of Chi-square test with Yates correction. Bold font indicates statistical significance. ART, antiretroviral therapy. Because more than one illness was detected in patients, the numbers of all illness are greater than the total number.

of 126 patients (52.6%) received ART during their lifetime. The mean age at death of patients on ART was 47.3 years, which was significantly higher than that of ART naïve patients (42.6 years; $P = 0.028$; Mann–Whitney *U*-test). Mean CD4 counts of ART (−) and (+) patients at the last blood examination before death were not significantly different (39.6 and 77.0 cells/μL, respectively, $P = 0.63$, Mann–Whitney *U*-test).

CMV was the most commonly identified pathogen among the autopsy cases (Table 2) and was detected in various organs, the most frequent being the adrenal gland (Figure 2A). PCP and NTM were also common pathogens found in the lungs of autopsied patients. *Candida albicans* was frequently detected in the gastrointestinal tract and oral cavity (Figure 2B). The prevalence of CMV and PCP was significantly lower in ART (+) patients than in ART (−) patients (Table 2). There was no significant difference in the prevalence of other opportunistic infections such as NTM and *Candida* or prevalence of HIV encephalopathy between ART (+) and (−) patients (Table 2).

Malignancies were identified in 50.2% (113/225) of all cases (Table 2). NHL was the most frequent malignancy with a lower prevalence in ART (−) patients (30.1%) than ART (+) patients (37.9%); however, the difference was not significant (Table 2). Diffuse large B-cell lymphoma was the most frequent histological subtype of NHL followed by Burkitt lymphoma, primary effusion lymphoma, and plasmablastic lymphoma (Table 3). Epstein–Barr virus positivity in lymphoma cases was significantly lower in ART (+) patients compared with ART (−) patients ($P = 0.001$, Chi-square test). KS was frequently found in the skin as well as other sites such as the gastrointestinal tract upon autopsy. In addition to NHL and KS, non-AIDS-defining malignancies such as Hodgkin lymphoma (HL), hepatic cancer, lung cancer, and leukemia were also observed in 20 patients. The prevalence of non-AIDS-defining malignancies was higher in ART (+) patients compared with ART (−) patients (Table 2).

The lung was the most frequent target for pathogens in patients with AIDS and 173 (76.9%) autopsy cases demonstrated the presence of lung-related illnesses (Table 4), which were significantly more frequent in ART (−) patients (112/136, 82.4%) than ART (+) patients (42/66, 63.6%) ($P = 0.003$, Chi-square test). CMV then PCP was the most frequently observed lung-related illnesses. The brain was the second most frequently affected organ in the autopsy cases. Although the brain was not investigated in 53 autopsies, 85 of the remaining 172 cases (49.4%) had brain-related illnesses, with CMV infection the most common, followed by lymphoma and HIV encephalopathy (Table 5). However, there was no significant difference in the rate of brain-related illnesses in ART (+) (37.8%, 17 of 45) or ART (−) patients (52.7%, 58/110) ($P = 0.091$, Chi-square test).

We also investigated the direct causes of death in the autopsied patients (Table 6). Lymphoma was the most frequent cause of death, followed by CMV infection. Non AIDS-defining cancers as a cause of death were significantly different between ART (−) (2, 1.5%) and ART (+) patients (6, 9.1%) ($P = 0.026$; Chi-square test with Yates correction). The prevalence of CMV, pneumonia, PCP, and NTM as a cause of death were lower in ART (+) patients compared with ART (−) patients, but no significant differences were observed between the groups.

Discussion

In the present study, we measured the prevalence of infectious disease and malignancy in autopsy cases of

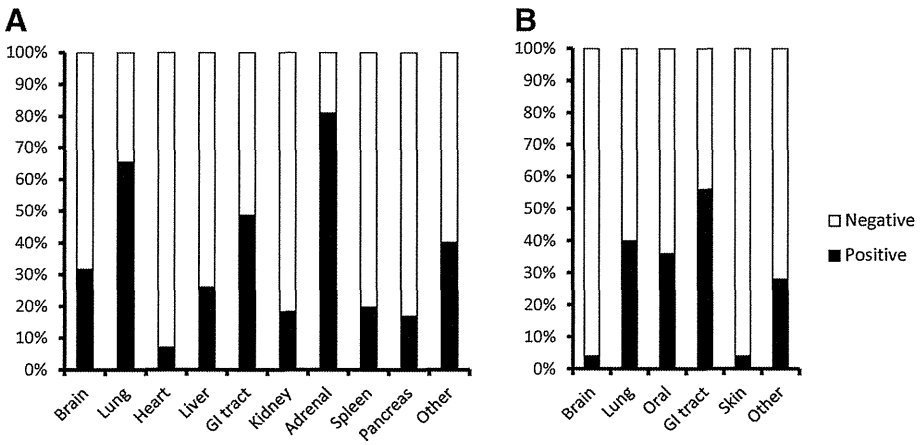

Figure 2 Distribution of cytomegalovirus and *Candida albicans*. (A) CMV positive rate in each organ. Black bar indicates the CMV positive rate in each organ from 142 CMV-positive patients. Because CMV was detected in more than one organ per patient, the sum of the black bars is over 100%. **(B)** The positive rate of *Candida albicans* in each organ. Black bar indicates the positive rate of *Candida albicans* per organ from 25 *Candida albicans*-positive patients.

Table 3 Non-Hodgkin lymphoma and Kaposi sarcoma in AIDS-associated autopsies

		Total		ART (–) patients		ART (+) patients		*P* values	
		n	%	n	%	n	%		
All NHL cases		71	100.0%	41	100.0%	25	100.0%		
Histology	DLBCL	53	74.6%	30	73.2%	18	72.0%	0.917	
	BL	4	5.6%	3	7.3%	1	4.0%	0.987	Y
	PEL	5	7.0%	4	9.8%	1	4.0%	0.706	Y
	PBL	1	1.4%	1	2.4%	0	0.0%	0.801	Y
	Other	6	8.5%	2	4.9%	4	16.0%	0.279	Y
	Unknown	2	2.8%	1	2.4%	1	4.0%	0.703	Y
Site	Nodular	1	1.4%	0	0.0%	1	4.0%	0.801	Y
	Extranodular	45	63.4%	28	68.3%	12	48.0%	0.102	
	Both	21	29.6%	11	26.8%	10	40.0%	0.265	
	Unknown	4	5.6%	2	4.9%	2	8.0%	0.987	Y
PCNS	Yes	27	38.0%	18	43.9%	6	24.0%	0.103	
EBV	Positive	52	73.2%	35	85.4%	12	48.0%	**0.001**	
KSHV	Positive	6	8.5%	5	12.2%	1	4.0%	0.495	Y
Cause of death	Yes	50	70.4%	33	80.5%	16	64.0%	0.137	
All KS cases		38		22		10			
Site	Skin	32	84.2%	19	86.4%	9	90.0%	0.410	
	GI tract	27	71.1%	15	68.2%	8	80.0%	0.705	
	Lung	21	55.3%	11	50.0%	6	60.0%	0.799	
	Lymph node	20	52.6%	13	59.1%	6	60.0%	0.502	
	Other	16	42.1%	0	0.0%	0	0.0%	0.787	Y
Cause of death	Yes	11	29.0%	7	31.8%	2	20.0%	0.791	Y

NHL, Non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; BL, Burkitt lymphoma; PEL, primary effusion lymphoma; PBL, plasmablastic lymphoma; PCNS, primary central nervous system lymphoma; EBV, Epstein–Barr virus; KSHV, Kaposi sarcoma-associated herpes virus; KS, Kaposi sarcoma; GI, gastrointestinal. *P* values were calculated by Chi-square test. Y indicates the use of Chi-square test with Yates correction. Bold font indicates statistical significance.

Table 4 Lung disease in patients infected with HIV

Illness	n	% of total patients (n = 225)
Any illness	173	76.9%
Cytomegalovirus infection	93	41.3%
Pneumocystis jirovecii pneumonia	66	29.3%
Any bacterial pneumonia	31	13.8%
Aspergillus infection	23	10.2%
Kaposi sarcoma	21	9.3%
Non-tuberculous mycobacterium infection	14	6.2%
Cryptococcosis	11	4.9%
Candida infection	10	4.4%
Tuberculosis	4	1.8%

Because more than one illness was detected in patients, the numbers of all illness are greater than the total number.

HIV-infected patients identified from 1985–2012 at four central hospitals in Japan. CMV infection, PCP, NTM infection, NHL, and KS were frequently observed in the autopsy cases. The prevalence of CMV and PCP was lower in ART (–) patients compared with ART (+) patients. The prevalence of non-AIDS defining malignancies was higher among ART (+) patients than ART (–) patients, suggesting that the onset of various opportunistic infections and malignancies should be carefully monitored regardless of whether the patient is receiving ART.

The autopsy cases in the present study were predominantly male (95.1%, Table 1). Additionally, more than 70% of the autopsy cases in the present study had a CD4 count < 200 cells/μL at the last blood examination before death (Table 1). A recent clinical study demonstrated the incidence of AIDS-defining illnesses in patients with HIV infection was decreased by the introduction of ART, especially in patients with CD4 counts >200 cells/μL [2]. Thus, our findings at autopsy cannot be compared with previous clinical studies because many clinical study patients had a high range of CD4 counts and ART responses. Interestingly, there was no significant difference in the

Table 5 Brain disease in patients infected with HIV

Illness	n	% in total autopsied brains (n = 172)
Any illness	85	49.4%
Cytomegalovirus infection	45	26.1%
Malignant lymphoma	26	15.1%
HIV encephalopathy	21	12.2%
Progressive multifocal leukoencephalopathy	8	4.7%
Toxoplasmosis	8	4.7%
Non-tuberculous mycobacterium infection	4	2.3%
Aspergillus infection	2	1.2%
Varicella zoster virus infection	2	1.2%
Herpes simplex virus infection	1	0.6%
Glioblastoma	1	0.6%
Candida infection	1	0.6%

Because more than one illness was detected in patients, the numbers of all illness are greater than the total number.

cause of death between ART (+) and (−) patients, with the exception of those with cancer (Table 6), indicating the prevalence of lethal illness did not differ between ART (+) and (−) patients.

Malignancies were frequent causes of death in the present study regardless of ART status (Table 6). Several studies demonstrated that the introduction of ART reduced the incidence of NHL in patients with HIV infection [13,15,19-23]. The use of ART has also been associated with a decrease in the incidence of KS [15,24,25]. However, an association between the incidence of non-AIDS-defining cancers and ART remains controversial. An increase of non-AIDS-defining cancers in patients receiving ART was shown in previous clinical reports [26,27], but a separate study showed that, with the exception of long-term protease inhibitor usage, ART exposure was generally not associated with a risk of non-AIDS-defining cancers [28]. The reasons for increased risk of non-AIDS-defining cancers in patients on ART are unclear, but might reflect the concomitant increase of the mean age at autopsy during the study period. This suggests that life extension of HIV-infected patients by ART results in the increased chance of developing non-AIDS events and malignancies. It was also

Table 6 Cause of death in AIDS-associated autopsies

	All		ART (−) patients		ART (+) patients			
	n	%	n	%	n	%	*P* values	
Total*	225	100.0%	136	100.0%	66	100.0%		
Malignant lymphoma	50	22.2%	33	24.3%	16	24.2%	0.997	
Cytomegalovirus	44	19.6%	27	19.9%	9	13.6%	0.279	
Pneumonia	31	13.8%	19	14.0%	9	13.6%	0.949	
Pneumocystis jirovecii pneumonia	30	13.3%	21	15.4%	4	6.1%	0.058	
Non-tuberculous mycobacterium	12	5.3%	10	7.4%	2	3.0%	0.367	Y
Kaposi sarcoma	11	4.9%	7	5.1%	2	3.0%	0.749	Y
Progressive multifocal leukoencephalopathy	8	3.6%	4	2.9%	2	3.0%	0.684	Y
Cancer	8	3.6%	2	1.5%	6	9.1%	**0.026**	Y
Hepatitis	8	3.6%	3	2.2%	4	6.1%	0.320	Y
Cryptococcus	7	3.1%	6	4.4%	0	0.0%	0.197	Y
Kidney failure	7	3.1%	4	2.9%	3	4.5%	0.861	Y
HIV encephalopathy	7	3.1%	5	3.7%	2	3.0%	0.861	Y
Aspergillus	6	2.7%	5	3.7%	0	0.0%	0.274	Y
Toxoplasmosis	4	1.8%	3	2.2%	1	1.5%	0.835	Y
Tuberculosis	3	0.9%	2	1.5%	0	0.0%	0.816	Y
Sepsis	3	1.3%	2	1.5%	1	1.5%	0.551	Y
Candida	3	1.3%	2	1.5%	0	0.0%	0.816	Y
Varicella zoster virus	2	1.3%	1	0.7%	1	1.5%	0.816	Y
Nocardia	1	0.4%	1	0.7%	0	0.0%	0.711	Y
Histoplasma	1	0.4%	1	0.7%	0	0.0%	0.711	Y

*Because more than one illness was detected in patients, the numbers of all illness are greater than the total number.
HIV, human immunodeficiency virus. *P* values were calculated with the Chi-square test. Y indicates the use of Chi-square test with Yates correction. Bold font indicates statistical significance.

demonstrated that ART introduction changed the pathological features of lymphoma; for example, a decrease of Epstein–Barr virus-positive lymphoma in Japanese patients with AIDS was reported [29]. Although HL was rare in the general Japanese population compared with European countries and the United States [30], the incidence of HL increased in Japanese patients on ART [17]. Thus, the increased risk of malignancies during the clinical course of HIV infection in patients receiving ART was reflected as a cause of death in the autopsy cases used in our study.

The prevalence of opportunistic infections differs among various regions and countries. In sub-Saharan African countries, more than 80% of HIV-positive patients die of infectious diseases, with disseminated tuberculosis being the most common (36%) [31]. Furthermore, there was no difference in the type of disease HIV patients succumbed to, regardless of ART status. In the USA and European countries, tuberculosis/NTM represented <10% of mortality in autopsy cases after 1996 [18]. In this study, tuberculosis was detected in only 2.7% of Japanese autopsy cases, but was the cause of death for 50% of afflicted patients. Mortality by PCP has decreased worldwide in patients with AIDS owing to prophylactic administration of an anti-PCP drug [16]. PCP was found in 36.4% (36/99 cases) of patients with AIDS before 1997, but was significantly reduced after 1997 (23.8%; 30/126 cases; $P = 0.04$; Chi-square test). This suggests that the decrease in PCP cases is associated with ART and anti-PCP prophylaxis.

Our study had several limitations. Bacterial culture was not available in this study owing to the use of formalin-fixed paraffin-embedded samples, and it was therefore difficult to identify the bacterial species responsible for many cases of pneumonia. Additionally, clinical information was limited. Information on HIV-RNA, an important indicator of ART effects, was not available for these patients. In addition, information regarding CD4 counts and the type, duration and possible interruption of ART were not available for a subset of patients. Therefore, we could not identify cases of immune reconstitution syndrome. Age at seroconversion and time living with HIV are also major predictors of HIV disease progression, however information of these parameters was limited. Thus, it should be noted that the conclusions in this study cannot be generally applied to the current HIV positive population in Japan. Furthermore, all findings in this study were obtained from autopsies.

Conclusions

Although further studies are required to demonstrate the association between ART and illness identified at autopsy, the present study demonstrates the prevalence of infectious diseases and malignancies in autopsy cases of HIV infection in Japan. While the prevalence of CMV infection and PCP at autopsy were lower in ART (+) patients than ART (−) patients, non-AIDS–defining malignancies were observed as a cause of death more frequently in ART (+) patients than ART (−) patients.

Abbreviations

HIV: Human immunodeficiency virus; ART: Antiretroviral therapy; AIDS: Acquired immunodeficiency syndrome; PCP: *Pneumocystis jirovecii* pneumonia; CMV: Cytomegalovirus; NTM: Non-tuberculous mycobacteria; NHL: Non-Hodgkin lymphoma; KS: Kaposi sarcoma.

Competing interests

The authors declare no conflicts of interests.

Authors' contributions

HK, S Okada and AY conceived this study; TH, MM, YKod, NO, YO, SM, TI, HH, and HK performed the autopsies, pathological analyses and reviews; AA, KT, JT, YKi, TU, TS, TK, AI, and S Oka collected clinical data; HK analyzed the data, performed statistical analyses, and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was partly supported by Health and Labour Sciences Research Grants [No. H22-AIDS-I-002, H23-AIDS-I-002, H24-AIDS-I-003, and H25-AIDS-I-002] from the Ministry of Health, Labour and Welfare and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan [No. 24659212 to HK]. The authors are indebted to the following investigators for contributing clinical data and providing excellent suggestions: Drs. Nobuaki Funata, Yuuko Yamada, Fumiaki Imamura, Akihiko Suganuma, Naoki Yanagisawa, Tokyo Metropolitan Komagome Hospital; Mr. Toshio Kitazawa, Department of Pathology, National Center for Global Health and Medicine Hospital; Dr. Takashi Odawara, Department of Infectious Diseases, Institute of Medical Science, University of Tokyo; Drs. Yasuharu Nishida, Dai Watanabe, Keishiro Yajima, Yoshihiko Ogawa, Yusuke Koizumi, Kazuyuki Hirota, Hitoshi Yonemoto, Hiroshi Otera, Daisuke Kasai, Motoko Ikuma, Masayuki Mano, Kiyoshi Mori, and Yukiko Morinaga, Osaka National Hospital.

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Received: 17 October 2013 Accepted: 25 April 2014
Published: 29 April 2014

References

1. Bruno R, Sacchi P, Filice G: **Overview on the incidence and the characteristics of HIV-related opportunistic infections and neoplasms of the heart: impact of highly active antiretroviral therapy.** *AIDS* 2003, **17**(Suppl 1):S83–S87.
2. Mocroft A, Furrer HJ, Miro JM, Reiss P, Mussini C, Kirk O, Abgrall S, Ayayi S, Bartmeyer B, Braun D, Castagna A, d'Arminio Monforte A, Gazzard B, Gutierrez F, Hurtado I, Jansen K, Meyer L, Munoz P, Obel N, Soler-Palacin P,

Papadopoulos A, Raffi F, Ramos JT, Rockstroh JK, Salmon D, Torti C, Warszawski J, de Wit S, Zangerle R, Fabre-Colin C, *et al*: **The Incidence of AIDS-Defining Illnesses at a Current CD4 Count > =200 Cells/μL in the Post-Combination Antiretroviral Therapy Era.** *Clin Infect Dis* 2013, **57**:1038–1047.

3. Mocroft A, Katlama C, Johnson AM, Pradier C, Antunes F, Mulcahy F, Chiesi A, Phillips AN, Kirk O, Lundgren JD: **AIDS across Europe, 1994–98: the EuroSIDA study.** *Lancet* 2000, **356**:291–296.
4. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD: **Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators.** *N Engl J Med* 1998, **338**:853–860.
5. Egger M, Hirschel B, Francioli P, Sudre P, Wirz M, Flepp M, Rickenbach M, Malinverni R, Vernazza P, Battegay M: **Impact of new antiretroviral combination therapies in HIV infected patients in Switzerland: prospective multicentre study. Swiss HIV Cohort Study.** *BMJ* 1997, **315**:1194–1199.
6. Young J, Psichogiou M, Meyer L, Ayayi S, Grabar S, Raffi F, Reiss P, Gazzard B, Sharland M, Gutierrez F, Obel N, Kirk O, Miro JM, Furrer H, Castagna A, De Wit S, Munoz J, Kjaer J, Grarup J, Chene G, Bucher H: **CD4 cell count and the risk of AIDS or death in HIV-Infected adults on combination antiretroviral therapy with a suppressed viral load: a longitudinal cohort study from COHERE.** *PLoS Med* 2012, **9**:e1001194.
7. Buchacz K, Baker RK, Palella FJ Jr, Chmiel JS, Lichtenstein KA, Novak RM, Wood KC, Brooks JT, HOPS-Investigators: **AIDS-defining opportunistic illnesses in US patients, 1994–2007: a cohort study.** *AIDS* 2010, **24**:1549–1559.
8. Ives NJ, Gazzard BG, Easterbrook PJ: **The changing pattern of AIDS-defining illnesses with the introduction of highly active antiretroviral therapy (HAART) in a London clinic.** *J Infect* 2001, **42**:134–139.
9. Silverberg MJ, Chao C, Leyden WA, Xu L, Tang B, Horberg MA, Klein D, Quesenberry CP Jr, Towner WJ, Abrams DI: **HIV infection and the risk of cancers with and without a known infectious cause.** *AIDS* 2009, **23**:2337–2345.
10. Yanik EL, Napravnik S, Cole SR, Achenbach CJ, Gopal S, Olshan A, Dittmer DP, Kitahata MM, Mugavero MJ, Saag M, Moore RD, Mayer K, Mathews WC, Hunt PW, Rodriguez B, Eron JJ: **Incidence and timing of cancer in HIV-infected individuals following initiation of combination antiretroviral Therapy.** *Clin Infect Dis* 2013, **57**:756–764.
11. Guiguet M, Boue F, Cadranel J, Lang JM, Rosenthal E, Costagliola D: **Effect of immunodeficiency, HIV viral load, and antiretroviral therapy on the risk of individual malignancies (FHDH-ANRS CO4): a prospective cohort study.** *Lancet Oncol* 2009, **10**:1152–1159.
12. Reekie J, Kosa C, Engsig F, Monforte A, Wiercinska-Drapalo A, Domingo P, Antunes F, Clumeck N, Kirk O, Lundgren JD, Mocroft A: **Relationship between current level of immunodeficiency and non-acquired immunodeficiency syndrome-defining malignancies.** *Cancer* 2010, **116**:5306–5315.
13. Clifford GM, Polesel J, Rickenbach M, Dal Maso L, Keiser O, Kofler A, Rapiti E, Levi F, Jundt G, Fisch T, Bordoni A, De Weck D, Franceschi S: **Cancer risk in the Swiss HIV Cohort Study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy.** *J Natl Cancer Inst* 2005, **97**:425–432.
14. Lim P-L, Zhou J, Ditangco R, Law M, Sirisanthana T, Kumarasamy N, Chen Y-M, Phanuphak P, Lee CKC, Saphonn V, Oka S, Zhang F, Choi J, Pujari S, Kamarulzaman A, Li PCK, Merati T, Yunihastuti E, Messerschmidt L, Sungkanuparph S: **Failure to prescribe pneumocystis prophylaxis is associated with increased mortality, even in the cART era: results from the Treat Asia HIV observational database.** *J Int AIDS Soc* 2012, **15**:1–1.
15. Yotsumoto M, Hagiwara S, Ajisawa A, Tanuma J, Uehira T, Nagai H, Fujikawa Y, Maeda S, Kitano K, Arima N, Uno K, Iwai T, Hongo I, Ota Y, Fukutake K, Okada S: **Clinical characteristics of human immunodeficiency virus-associated Hodgkin lymphoma patients in Japan.** *Int J Hematol* 2012, **96**:247–253.
16. Hashimoto S, Matsumoto T, Nagai M, Matsuyama Y, Nakamura Y, Umeda T, Kamakura M, Ichikawa S, Kimura S, Fukutomi K, Kihara M: **Delays and continuation of hospital visits among HIV-infected persons and AIDS cases in Japan.** *J Epidemiol* 2000, **10**:65–70.
17. Nakamura H, Teruya K, Takano M, Tsukada K, Tanuma J, Yazaki H, Honda H, Honda M, Gatanaga H, Kikuchi Y, Oka S: **Clinical symptoms and courses of primary HIV-1 infection in recent years in Japan.** *Intern Med* 2011, **50**:95–101.
18. Masliah E, DeTeresa RM, Mallory ME, Hansen LA: **Changes in pathological findings at autopsy in AIDS cases for the last 15 years.** *AIDS* 2000, **14**:69–74.
19. Tirelli U, Spina M, Gaidano G, Vaccher E, Franceschi S, Carbone A: **Epidemiological, biological and clinical features of HIV-related lymphomas in the era of highly active antiretroviral therapy.** *AIDS* 2000, **14**:1675–1688.
20. Kirk O, Pedersen C, Cozzi-Lepri A, Antunes F, Miller V, Gatell JM, Katlama C, Lazzarin A, Skinhoj P, Barton SE: **Non-Hodgkin lymphoma in HIV-infected patients in the era of highly active antiretroviral therapy.** *Blood* 2001, **98**:3406–3412.
21. Carrieri MP, Pradier C, Piselli P, Piche M, Rosenthal E, Heudier P, Durant J, Serraino D: **Reduced incidence of Kaposi's sarcoma and of systemic non-hodgkin's lymphoma in HIV-infected individuals treated with highly active antiretroviral therapy.** *Int J Cancer* 2003, **103**:142–144.
22. Vilchez RA, Jorgensen JL, Kroll MH: **Systemic non-Hodgkin lymphoma in HIV-infected patients in the era of highly active antiretroviral therapy.** *Blood* 2002, **99**:4250–4251.
23. Wolf T, Brodt HR, Fichtlscherer S, Mantzsch K, Hoelzer D, Helm EB, Mitrou PS, Chow KU: **Changing incidence and prognostic factors of survival in AIDS-related non-Hodgkin's lymphoma in the era of highly active antiretroviral therapy (HAART).** *Leuk Lymphoma* 2005, **46**:207–215.
24. Pipkin S, Scheer S, Okeigwe I, Schwarcz S, Harris DH, Hessol NA: **The effect of HAART and calendar period on Kaposi's sarcoma and non-Hodgkin lymphoma: results of a match between an AIDS and cancer registry.** *AIDS* 2011, **25**:463–471.
25. Franceschi S, Lise M, Clifford GM, Rickenbach M, Levi F, Maspoli M, Bouchardy C, Dehler S, Jundt G, Ess S, Bordoni A, Konzelmann I, Frick H, Dal Maso L, Elzi L, Furrer H, Calmy A, Cavassini M, Ledergerber B, Keiser O: **Changing patterns of cancer incidence in the early- and late-HAART periods: the Swiss HIV Cohort Study.** *Br J Cancer* 2010, **103**:416–422.
26. Powles T, Robinson D, Stebbing J, Shamash J, Nelson M, Gazzard B, Mandelia S, Moller H, Bower M: **Highly active antiretroviral therapy and the incidence of non-AIDS-defining cancers in people with HIV infection.** *J Clin Oncol* 2009, **27**:884–890.
27. Hagiwara S, Yotsumoto M, Odawara T, Ajisawa A, Uehira T, Nagai H, Tanuma J, Okada S: **Non-AIDS-defining hematological malignancies in HIV-infected patients: an epidemiological study in Japan.** *AIDS* 2013, **27**:279–283.
28. Chao C, Leyden WA, Xu L, Horberg MA, Klein D, Towner WJ, Quesenberry CP Jr, Abrams DI, Silverberg MJ: **Exposure to antiretroviral therapy and risk of cancer in HIV-infected persons.** *AIDS* 2012, **26**:2223–2231.
29. Hishima T, Oyaizu N, Fujii T, Tachikawa N, Ajisawa A, Negishi M, Nakamura T, Iwamoto A, Hayashi Y, Matsubara D, Sasao Y, Kimura S, Kikuchi Y, Teruya K, Yasuoka A, Oka S, Saito K, Mori S, Funata N, Sata T, Katano H: **Decrease in Epstein-Barr virus-positive AIDS-related lymphoma in the era of highly active antiretroviral therapy.** *Microbes Infect* 2006, **8**:1301–1307.
30. Levi F, Lucchini F, Negri E, Boyle P, La Vecchia C: **Trends in mortality from Hodgkin's disease in Western and Eastern Europe.** *Br J Cancer* 2002, **87**:291–293.
31. Cox JA, Lukande RL, Nelson AM, Mayanja-Kizza H, Colebunders R, Van Marck E, Manabe YC: **An autopsy study describing causes of death and comparing clinico-pathological findings among hospitalized patients in Kampala, Uganda.** *PLoS One* 2012, **7**:e33685.

doi:10.1186/1471-2334-14-229
Cite this article as: Katano *et al.*: The prevalence of opportunistic infections and malignancies in autopsied patients with human immunodeficiency virus infection in Japan. *BMC Infectious Diseases* 2014 **14**:229.

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 30, Number 6, 2014

DOI: 10.1089/aid.2013.0252

Long-Term Use of Protease Inhibitors Is Associated with Bone Mineral Density Loss

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Abstract

HIV-infected patients are at high risk for bone mineral density (BMD) loss. The present study was designed to provide information on characteristics of BMD abnormalities in Japanese HIV-1-infected patients and risk factors involved in worsening of BMD. A total of 184 Japanese HIV-1-infected men were studied with a dual-energy X-ray absorptiometry scan (DXA) at the lumbar spine and femoral neck. Multivariate logistic regression models were used for comparison of the impact of risk factors on BMD loss. Osteopenia and osteoporosis were diagnosed in 46% and 10% of the patients at lumbar spine, and 54% and 12% at femoral neck, respectively. In logistic analysis, factors associated with low BMD at both lumbar spine and femoral neck were long-term treatment with a protease inhibitor (PI) [odds ratio (OR) 1.100 and 1.187 per 1 year increase of PI use; 95% confidence interval (CI) 1.003–1.207 and 1.043–1.351; $p=0.042$ and 0.009, respectively] and a low body mass index [OR: 0.938 and 0.852, CI 0.892–0.992 and 0.783–0.927; $p=0.024$ and <0.001, respectively]. Patients who discontinued PI had a significantly higher BMD than those who currently use PI at lumbar spine (t score -0.8 vs. -1.3, $p=0.04$) but not at femoral neck (-1.3 vs. -1.5, $p=0.38$). In HIV-infected Japanese patients, the duration of treatment with PI correlated significantly with BMD loss. Discontinuation of PI is a promising option in the treatment of BMD loss since it allows recovery of BMD, especially in the lumbar spine.

Introduction

For HIV-infected patients, loss of bone mineral density (BMD) is an important age-related complication, in addition to chronic renal dysfunction, cardiovascular diseases, and metabolic disorders. A meta-analysis study reported that the prevalence of osteoporosis among HIV-infected patients was three times higher than in the HIV-negative population.[1] The etiology of low BMD in HIV-infected patients is multifactorial and is considered to include chronic HIV infection[2,3] and antiretroviral therapy, especially tenofovir disoproxil fumarate (TDF) and protease inhibitors (PI).[4–7] However, to our knowledge, information on the characteristics of BMD abnormalities in Asian HIV-infected patients is scarce and the exact risk factors involved in the worsening of BMD remain obscure. The present study was designed to provide new information on the above two aspects of Asian HIV-1 infection.

Materials and Methods

Setting and participants

We performed a cross-sectional study at the AIDS Clinical Center (ACC), National Center for Global Health and Medicine (NCGM) involving HIV-infected patients who were registered at the NCGM from February 2012 to June 2013. We excluded patients who had been on treatment for osteoporosis, current users of corticosteroids, and those with a history of bone fractures at the spine or bilateral femoral neck. A total of 184 Japanese HIV-infected men were enrolled in this study. This study was approved by the ethics committee of NCGM and a written informed consent was obtained from each patient.

Data collection

BMD was assessed using dual X-ray absorptiometry (DXA: QDR-4500W, Hologic Inc., Bedford, MA) at the lumbar spine

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and femoral neck. Osteopenia and osteoporosis were defined using the World Health Organization (WHO) criteria. Normal BMD was defined as a *t* score of −1 or higher, osteopenia as a *t* score between −1 and −2.5, and osteoporosis as a *t* score of −2.5 or lower.[8] Age, body mass index (BMI), smoking habit, hemophilia, history of an AIDS-defined illness, nadir CD4 cell count, time with low CD4 cell count (<200 cell/μl), time on antiretroviral therapy (ART), TDF, and PI, were obtained by interview or medical records. Estimated glomerular filtration rate (eGFR) was calculated using the modified Modification of Diet in Renal Disease (MDRD) equation for Japanese populations.[9]

Statistical analysis

t scores and BMD of the lumbar spine and femoral neck were compared using Student's paired *t*-test. To determine the impact of independent variables, multivariate logistic regression analysis was used. In logistic regression analysis, the dependent variable was set as low BMD (*t* score lower than −1.0) at both the lumbar spine and femoral neck. We used the odds ratio (ORs) and 95% confidence interval (95% CI) to estimate the impact of each variable on low BMD.

To assess the impact of PI discontinuation, we compared the *t* scores between PI-experienced patients and patients who discontinued such therapy, using the Student's unpaired *t*-test. For evaluation of the correlation between the *t* score at the lumbar spine and the time on PI, ritonavir (RTV) at different dosage (100 mg/day and 200 mg/day), and other types of PI, Pearson's correlation coefficient was used. For further evaluation of the relationship between the time on TDF and BMD, we compared the *t* scores between those who were treated with PI plus TDF and those treated with PI only and had never been treated with TDF, using the Student's unpaired *t*-test. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Patient characteristics

The characteristics of the 184 study patients are summarized in Table 1. All patients underwent DXA for the lumbar spine and 164 underwent DXA for the femoral neck. Hemophiliacs constituted 36% ($n=67$) of the study subjects. Seventy-one patients (39%) had a history of infection with hepatitis C virus (HCV), including all 67 hemophiliacs. Among them, 16 of the 71 (23%) patients showed spontaneous viral clearance, 23 (32%) achieved sustained virologic response after antiviral therapy, and 2 (3%) patients were still on treatment and had undetectable levels of HCV viral load. The remaining 30 (45%) patients with chronic hepatitis C were nonresponders or never users of antiviral therapy. Among them, 9 (14%) had liver cirrhosis diagnosed by radiological findings. Although 41 (21%) patients had a history of AIDS-defined illness, 172 (93%) patients had been treated with ART and 148 (80%) patients had an undetectable level of HIV viral load.

The median durations of ART, PI, and TDF of the total population were 88, 38, and 23 months, respectively. Among 139 TDF-treated patients, the median time on TDF was 38 months (IQR 14–68 months). One hundred and forty-four patients had previously received PI-based treatment, and the numbers of patients who had been treated with each type of PI were 30 patients with nelfinavir (NFV), 47 with lopinavir (LPV/r), 34 with atazanavir (ATV), 21 with fosamprenavir (FPV) or amprenavir (APV), 74 with darunavir (DRV), 4 with indinavir (IDV), and 1 with saquinavir (SQV). The total number of patients who had received RTV was 137, and of these, 102 and 63 patients had been treated with RTV at 100 and 200 mg/day, respectively.

TABLE 1. CLINICOPATHOLOGICAL CHARACTERISTICS OF THE 184 STUDY PATIENTS

Sex, (male/female)	184/0
Age: median (IQR)	43 (38–51)
Body mass index (kg/m^2)	22 (20–24)
Hypertension, *n* (%)	42 (23%)
Current smoking, *n* (%)	99 (54%)
Hemophilia, *n* (%)	67 (36%)
History of AIDS-defined illness, *n* (%)	40 (22%)
Positive HBsAg, *n* (%)	8 (4%)
Positive HCV-Ab, *n* (%)	71 (37%)
Liver cirrhosis, *n* (%)	10 (5%)
Diabetes mellitus, *n* (%)	7 (4%)
Current CD4$^+$ T cell count (cells/μl)	493 (322–623)
Nadir CD4$^+$ T cell count (cells/μl)	141 (54–218)
Low CD4$^+$ T cell count (<200 cells/μl) for >1 year, *n* (%)	52 (28%)
Current suppressed viral load (<20 copies/ml), *n* (%)	148 (80%)
Current use of ART, *n* (%)	172 (93%)
Time on ART (months)	88 (26–153)
Current use of protease inhibitors, *n* (%)	117 (64%)
Never use of protease inhibitors, *n* (%)	40 (22%)
Time on protease inhibitors (months)	38 (2–81)
Current use of tenofovir, *n* (%)	114 (62%)
Never use of tenofovir, *n* (%)	45 (24%)
Time on tenofovir (months)	22 (0–60)
Serum creatinine (mg/dl)	0.78 (0.68–0.89)
Estimated glomerular filtration rate (ml/min/1.73 m^2)	86.0 (74.7–100.3)

Values are median (IQR) or number (%) of patients.
HBsAg, hepatitis B virus surface antigen; HCV-Ab, hepatitis C virus antibody; ART, antiretroviral therapy; ART, antiretroviral therapy.

Prevalence of low bone mineral density

Based on the WHO criteria, osteopenia and osteoporosis were diagnosed in 46% and 10% of the patients at the lumbar spine and 53% and 12% at the femoral neck, respectively. The mean *t* scores were −1.1 [standard deviation (SD) 1.1] for the lumbar spine and −1.4 (SD: 1.1) for the femoral neck (Fig. 1A). The mean BMD scores were 0.914 g/cm^2 (SD: 0.199 g/cm^2) at the lumbar spine and 0.694 g/cm^2 (SD: 0.221 g/cm^2) at the femoral neck (Fig. 1B). Both the *t* score and BMD at the femoral neck were significantly lower than those at the lumbar spine ($p=0.008$ for *t* score and $p<0.001$ for BMD).

Impact of related risk factors

In multivariate logistic analysis, statistically significant regression models were built for low BMD (*t* score < −1) at the lumbar spine ($p=0.038$) and at the femoral neck

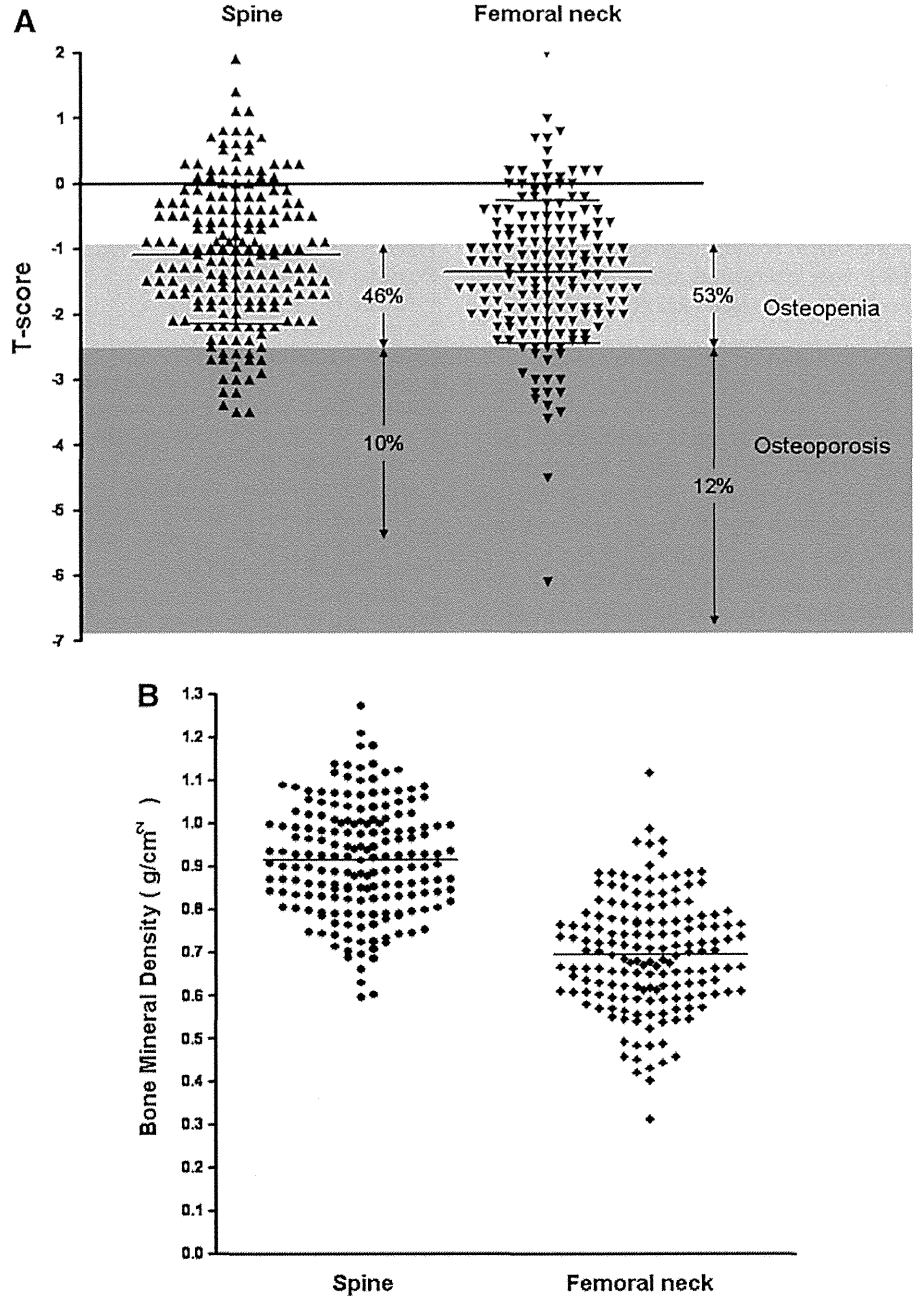

FIG. 1. (**A**) Distribution of *t* scores at lumbar spine and femoral neck. Light areas: osteopenia; dark gray areas: osteoporosis. (**B**) Distribution of bone mineral density (BMD) at lumbar spine and femoral neck. In both (**A**) and (**B**), data are mean ± standard deviation. Differences in the mean scores of the spine and femoral neck were tested by the Student's paired *t*-test.

($p < 0.001$) (Table 2). In logistic analysis, the following factors were associated with low BMD at both the lumbar spine and femoral neck: longer duration of treatment with a PI [odds ratio (OR) 1.100 and 1.187 per 1 year increase of PI use and 95% confidence interval (CI) 1.003–1.207 and 1.043–1.351; $p = 0.042$ and 0.009, respectively] and lower body mass index [OR: 0.938 and 0.852, CI 0.892–0.992 and 0.783–0.927; $p = 0.024$ and < 0.001, respectively]. Low BMD at the femoral neck also correlated with age [OR: 1.071; CI 1.029–1.115; $p = 0.001$] and hemophilia [OR: 8.139; CI 2.594–25.337; $p < 0.001$].

Impact of PI use and discontinuation on bone mineral density

The *t* scores of both the spine and femoral neck were significantly lower in patients who received PI than in those who never used PI [−1.2 vs. −0.7 at the spine ($p = 0.02$) and −1.5 vs. −0.9 at the femoral neck ($p = 0.002$), respectively] (Fig. 2A). Moreover, patients who discontinued PI had a higher spine *t* score than those who currently used PI (−0.8 vs. −1.3, $p = 0.04$) and had a *t* score level comparable to those patients who never used PI (−0.8 in PI-discontinued patients

TABLE 2. RESULTS OF LOGISTIC ANALYSIS FOR BONE MINERAL ABNORMALITIES MEASURED FOR DIFFERENT JOINTS

	Univariate analysis			*Multivariate analysis*[a]		
	OR	*95% CI*	p *value*	*OR*	*95% CI*	p *value*
Low BMD at lumbar spine (t score < -1.0)						
Age (per 1 year increase)	1.015	0.986–1.045	0.309	1.016	0.989–1.042	0.249
Body mass index (per 1 increase)	0.924	0.845–1.011	0.086	0.938	0.892–0.992	0.024
Hemophilia	1.013	0.556–1.847	0.967			
Current smoking	1.690	0.942–3.302	0.078	1.651	0.903–2.971	0.104
History of AIDS-defined illness	1.630	0.800–3.323	0.176			
Nadir CD4 (per 1 increase of categories)						
≥350	1.000					
200–349	0.514	0.140–1.883	0.315			
≤199	0.799	0.241–2.653	0.714			
Time with CD4 <200/μl (per 1 year increase)	1.065	0.921–1.233	0.515			
Time on ART (per 1 year increase)	1.027	0.978–1.077	0.287	0.973	0.912–1.038	0.408
Time on TDF (per 1 year increase)	1.082	0.976–1.200	0.134	1.078	0.961–1.210	0.201
Time on PI (per 1 year increase)	1.081	1.009–1.159	0.026	1.100	1.003–1.207	0.042
Low BMD at femoral neck (t-score < -1.0)						
Age (per 1 year increase)	1.012	1.005–1.019	0.001	1.071	1.029–1.115	0.001
Body mass index (per 1 increase)	1.017	1.003–1.031	0.018	0.852	0.783–0.927	<0.001
Hemophilia	3.954	1.850–8.448	<0.001	8.139	2.594–25.337	<0.001
Current smoking	1.206	0.642–2.265	0.561	0.238	0.734–3.460	0.238
History of AIDS-defined illness	1.870	0.806–4.338	0.141	0.124	0.795–6.789	0.124
Nadir CD4 (per 1 increase of categories)						
≥350	1.000			1.000		
200–349	1.593	0.425–5.971	0.489	1.553	0.355–6.783	0.559
≤199	0.984	0.293–3.301	0.979	0.757	0.174–3.285	0.710
Time with CD4 <200/μl (per 1 increase of categories)	1.072	0.951–1.209	0.257	0.844	0.684–1.042	0.114
Time on ART (per 1 year increase)	1.070	1.034–1.117	<0.001	0.968	0.880–1.066	0.509
Time on TDF (per 1 year increase)	1.084	1.005–1.119	0.037	0.990	0.848–1.156	0.900
Time on PI (per 1 year increase)	1.151	1.079–1.225	<0.001	1.187	1.043–1.351	0.009

[a]In the analysis for lumbar spine, the final model obtained by backward stepwise elimination included the time on ART, TDF, and PI, current smoking, BMI, and age. OR, odds ratios; CI, confidence intervals; ART, antiretroviral therapy; TDF, tenofovir disoproxil fumarate; PI, protease inhibitors; BMD, bone mineral density.

vs. −0.7 in PI-never use patients, $p=0.97$) (Fig. 2B). In contrast, there was no significant difference in femoral neck t score between PI-discontinued patients and PI current-use patients (−1.3 vs. −1.5, $p=0.38$) or between PI-discontinued patients and PI-never use patients (−1.3 vs. −0.9, $p=0.24$) (Fig. 2C).

Impact of different types of PIs on bone mineral density

While the correlation between the duration of treatment of any PI and spine t score was significant ($r=-0.180$, $p=0.013$) (Fig. 3A), the duration of treatment with RTV showed a better correlation with spine t score (−0.207, $p=0.004$) (Fig. 3B). When both the time on RTV and the time on PI were entered as independent variables in logistic analysis for low BMD at the lumbar spine, a statistically significant model was built by elimination of the time on PI. In this model, the time on RTV was significantly associated with low BMD (OR: 1.146, 95% CI 1.032–1.273, $p=0.011$). At the femoral neck, RTV was associated with low BMD (OR: 1.267 per 1 year increase of RTV, 95% CI 1.010–1.589, $p=0.041$), whereas the time on PI was not (OR: 0.983 per 1 year increase of PI, 95% CI 0.803–1.202, $p=0.864$). There were no significant correlations between spine t score and the duration of treatment with RTV at either 100 mg/day ($r=-0.134$, $p=0.071$) (Fig. 3C) or 200 mg/day ($r=-0.133$, $p=0.073$) (Fig. 3D). No significant correlations were found between different types of PIs and spine t score (NFV: $r=-0.023$, $p=0.758$; LPV/r: $r=-0.080$, $p=0.239$; DRV: $r=-0.069$, $p=0.355$; ATV: $r=-1.123$, $p=0.097$; FPV or APV: $r=0.091$, $p=0.218$).

Comparison of BMD between PI- and PI-TDF-treated patients

For further confirmation of the poor association between TDF use and BMD loss, t scores were compared between patients who had been treated with both PI and TDF ($n=118$) and patients who received PI-based treatment and had never been treated with TDF ($n=26$). Neither spine nor femoral neck t scores were significantly different between the two groups (PI + TDF: −1.2, PI alone: −1.0, $p=0.414$ for spine t score, −1.5 vs. −1.5, $p=0.844$ for femoral neck, respectively).

Discussion

The present study showed that for Asian HIV-infected patients, PI use was the most significant determinant of low BMD at both the spine and femoral neck. Moreover, our logistic regression models strongly suggested that long-term use of PI has a gradual and cumulative effect on BMD.

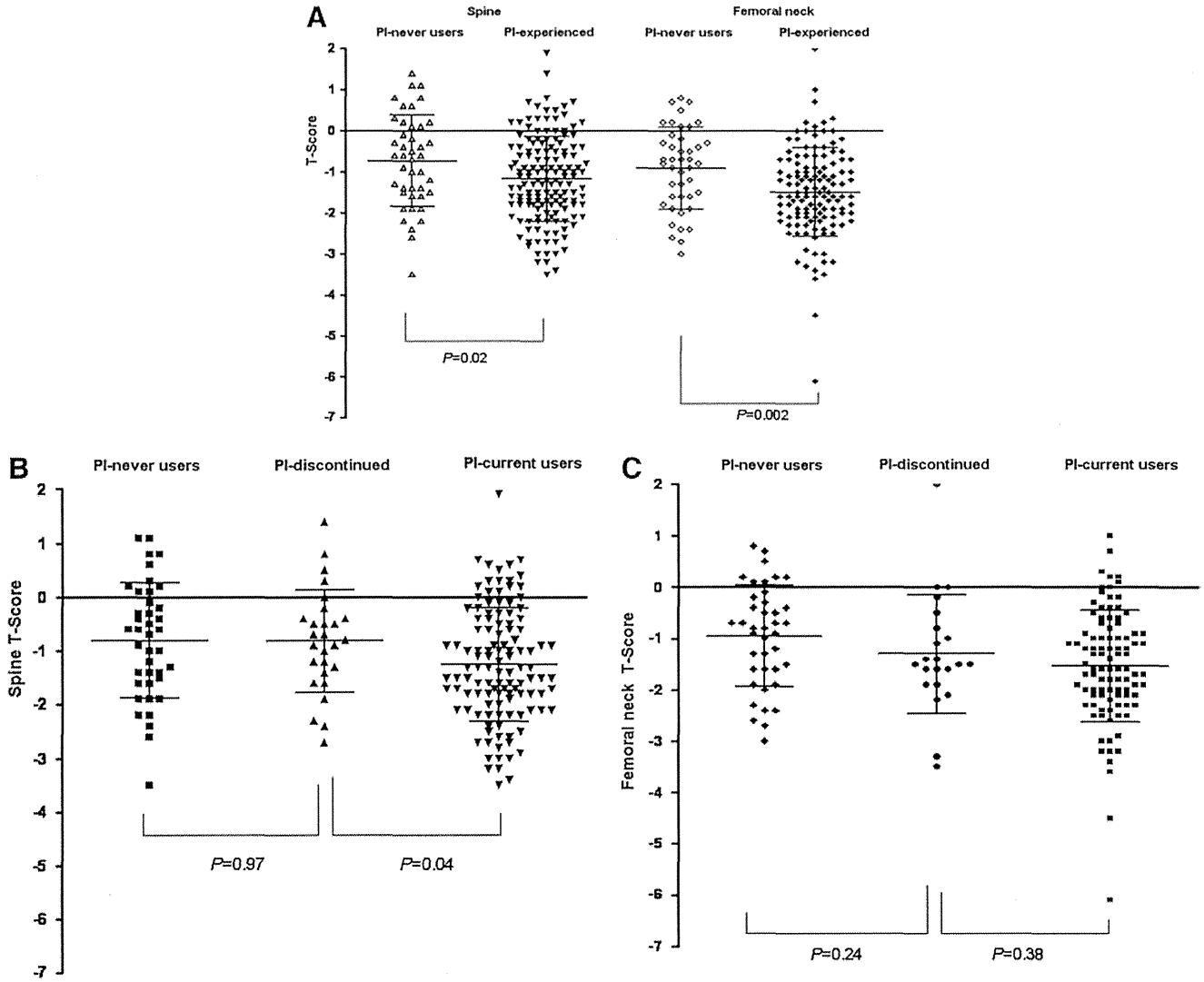

FIG. 2. (**A**) Comparison of *t* scores at lumbar spine and femoral neck between patients who were treated and never treated with a protease inhibitor (PI). Comparison of *t* score at lumbar spine (**B**) and at femoral neck (**C**) among patients who never used PI (left), discontinued PI (center), and are currently using PI (right). Data are mean ± standard deviation.

Although large cohort studies have already shown that PI use can cause BMD loss,[4–6] it still remains unclear which type of PI causes BMD loss. Our study found no significant association between the use of any particular type of PI and BMD loss, which is consistent with a previous *in vitro* study that evaluated the impact of different PIs on osteoblast activity using an osteoblast-like cell line.[10] Both *in vitro*[11,12] and *ex vivo* studies[13] reported that RTV promotes the proliferation/activation of osteoclasts, causing increased bone absorption. Our study added support to previous studies that RTV plays a major role in PI-associated BMD loss,[13] although there is insufficient data to conduct direct a comparison of BMD between patients treated with unboosted and boosted PI. The correlations between the two different dosages of RTV and BMD were almost comparable levels of strength, suggesting that RTV can cause BMD loss not dose dependently but time dependently irrespective of the dose. However, at this stage, we recommend further evaluation of the effect of each type of PI, since the subanalyses conducted in the present study have limited power for cause–effect evaluation due to the relatively small number of patients treated with certain types of PI.

Does discontinuation of PI lead to recovery of BMD? It seems there is no definitive answer to this question. A small cohort substudy showed possible BMD recovery after switching PI to raltegravir.[14] However, the change in BMD after switching was too small in that study to confirm the recovery effect of PI discontinuation. The present study provides additional data in support of a lower decrease in BMD by showing a large difference in BMD between PI-discontinued and -continued patients, although it is a cross-sectional study. A prospective longitudinal cohort study using a larger population on longer use of PI is necessary for a more precise evaluation of the reversibility of PI-associated BMD loss. It should be noted that the PI-discontinued patients showed a higher BMD level not in the femoral neck but in the lumbar spine, which is consistent with some large cohort study showing that PI causes greater BMD loss in the lumbar spine than the femoral neck.[4,5] This interesting discrepancy is well explained by the difference in bone tissue

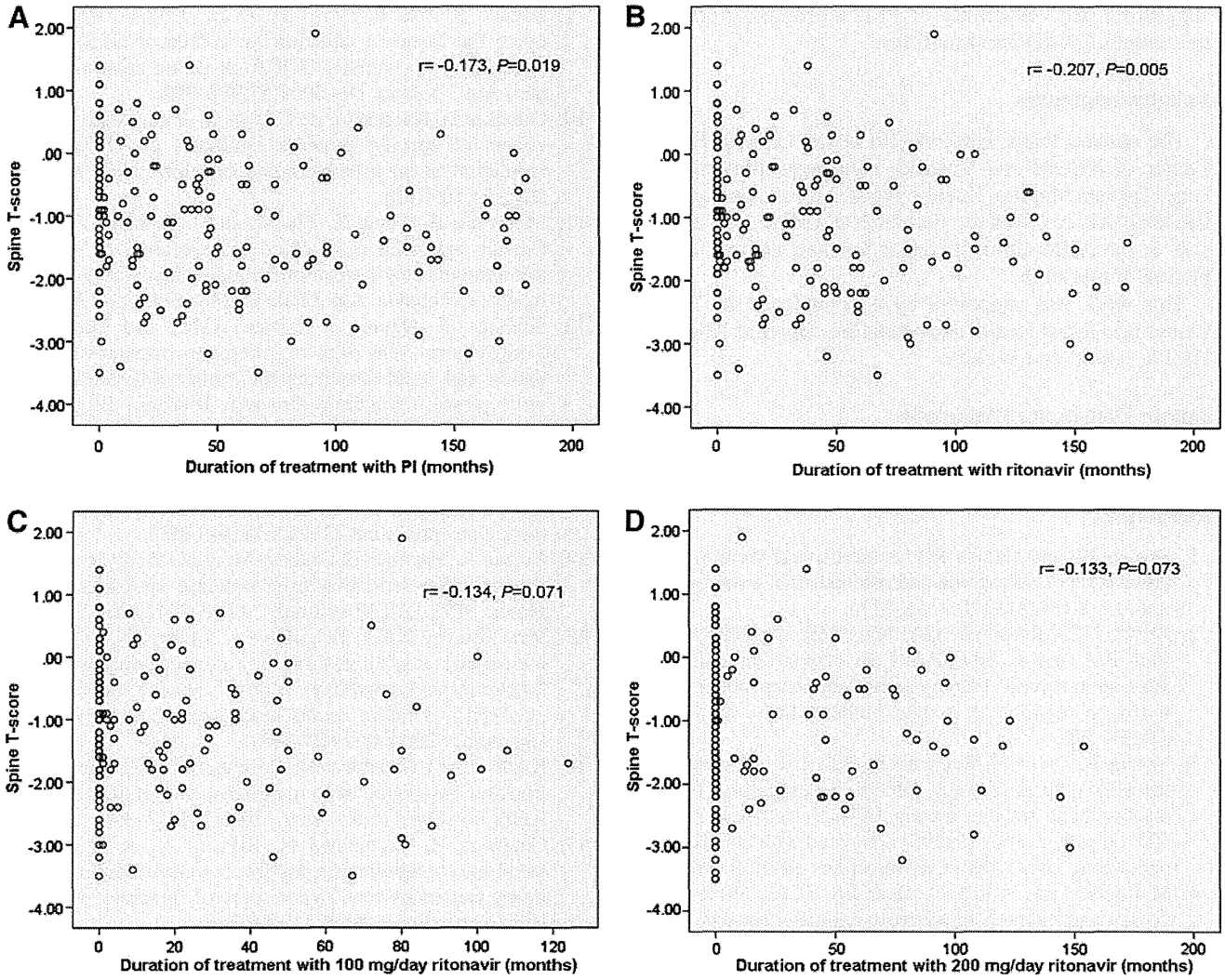

FIG. 3. Scattered dot plots of the correlation between *t* score at lumbar spine and duration of treatment with PI **(A)**, ritonavir (RTV) **(B)**, RTV at 100 mg/day **(C)**, and RTV at 200 mg/day **(D)**. Correlations were tested by Pearson's correlation coefficient.

type between the vertebrae and femur. While the femur contains abundant cortical substance with few osteoclasts, the vertebrae comprise osteoclast-rich trabecular substance. Therefore, discontinuation of osteoclast-activating agents, such as RTV, can cause a slower decrease of BMD in vertebrae compared with the femur.

TDF can cause BMD loss mainly through persistent urinary loss of phosphates.[4,7,15,16] However, our study did not show any significant association between TDF use and low BMD. While the exact reason for this finding is not clear, it could be related to the general clinical practice in Japan: TDF is often discontinued in Japan upon identification of modest proximal tubular dysfunction (a low level of percent tubular reabsorption of phosphates or a high level of urine-β_2-microglobulin) in HIV-infected patients.[16,17] This practice is an important limitation in the present study.

Hemophilia is a risk factor for BMD loss based on the associated hemophilic arthropathy and long-term disuse.[18,19] However, the present study demonstrated an almost equal prevalence of spine BMD abnormalities in hemophiliacs and HIV-infected patients [rate of osteoporosis, hemophiliacs: 5/67 (7%), other patients: 14/117 (12%); rate of osteopenia, hemophiliacs: 32/67 (48%), other patients: 52/117 (44%)]. Furthermore, the mean (standard deviation) *t* score of the lumbar spine was −1.1 (1.0) in hemophiliacs and −1.1 (1.1) in other patients. Thus, with regard to lumbar spine BMD, the present study well reflects the general Asian HIV-infected population. On the other hand, BMD abnormalities are common in hemophiliacs including abnormalities of the femoral neck [rate of osteoporosis, hemophiliacs: 15/57 (26%), other patients: 5/107 (5%); rate of osteopenia, hemophiliacs: 32/57 (56%), other patients: 56/107 (52%)]. The mean (standard deviation) *t* score of the femoral neck was −2.0 (1.1) in hemophiliacs and −1.0 (0.9) in other patients. Multivariate analysis identified age, BMI, and hemophilia as significant determinants of BMD at the femoral neck. Thus, BMD at the femoral neck is considered to be largely influenced by weight load and disuse.

In conclusion, long-term use of PI was identified as a significant risk factor for BMD loss in HIV-infected Asian patients. Furthermore, the results demonstrated that the negative effect of PI on BMD was time dependent. In particular, RTV plays a major role in PI-associated BMD loss irrespective of the dose. Discontinuation of PI seems to lessen the decrease in BMD, especially in the lumbar spine,

suggesting that withdrawal of PI is a promising option for treatment of BMD abnormalities.

Acknowledgments

The authors thank Hiroyuki Ito at the Clinical Research Center of NCGM and Yasuaki Yanagawa, Masahiro Ishikane, Takashi Matono, Kazuko Ikeda, Miwa Ogane, Michiyo Ishisaka, Akiko Nakano, Fumihide Kanaya, and all other staff at the AIDS Clinical Center for their help in the completion of this study.

This work was supported by a grant from the National Center for Global Health and Medicine, Japanese Ministry of Health, Labor, and Welfare.

Author Disclosure Statement

No competing financial interests exist.

References

1. Brown TT and Qaqish RB: Antiretroviral therapy and the prevalence of osteopenia and osteoporosis; a meta-analytic review. AIDS 2006;20:2165–2174.
2. Brown TT, McComsey GA, King MS, Qaqish RB, Bernstein BM, and da Silva BA: Loss of bone mineral density after antiretroviral therapy initiation, independent of antiretroviral regimen. J Acquir Immune Defic Syndr 2009; 51:554–561.
3. Young B, Dao CN, Buchacz K, Baker R, Brooks JT, and the HIV outpatient study (HOPS) investigators: Increased rates of bone fracture among HIV-infected persons in the HIV outpatient study (HOPS) compared with the US general population, 2000–2006. Clin Infect Dis 2011;52:1061–1068.
4. McComsey GA, Kitch D, Daar ES, *et al.:* Bone mineral density and fractures in antiretroviral-naïve persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: AIDS clinical trials group A5224s, a substudy of ACTG A5202. J Infect Dis 2011;203:1791–1801.
5. Duvivier C, Kolta S, Assoumou L, *et al.:* Greater decrease in bone mineral density with protease inhibitor regimens compared with non-nucleoside reverse transcriptase inhibitor regimens in HIV-1 infected naive patients. AIDS 2009; 23:817–824.
6. Bonjoch A, Figueras M, Estany C, *et al.:* High prevalence of and progression to low bone mineral density in HIV-infected patients: A longitudinal cohort study. AIDS 2010; 24:2827–2833.
7. Moyle GJ, Stellbrink HJ, Compston J, *et al.:* 96-week results of abacavir/lamivudine versus tenofovir/emtricitabine, plus efavirenz, in antiretroviral-naïve, HIV-1-infected adults: ASSERT study. Antivir Ther 2013;18:905–913.
8. Prevention and management of osteoporosis. World Health Organ Tech Rep Ser 2003;921:1–164
9. Matsuo S, Imai E, Horio M, *et al.:* Collaborators developing the Japanese equation for estimated GFR: Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982–992.
10. Gibellini D, Borderi M, de Crignis E, *et al.:* Analysis of the effects of specific protease inhibitors on OPG/RANKL regulation in an osteoblast-like cell line. New Microbiol 2010;33:109–115.
11. Modarresi R, Xiang Z, Yin M, and Laurence J: WNT/ β-Catenin signaling is involved in regulation of osteoclast differentiation by human immunodeficiency virus protease inhibitor ritonavir. Am J Pathol 2009;174:123–135.
12. Santiago F, Oguma J, Brown AMC, and Laurence J: Noncanonical Wnt signaling promotes osteoclast differentiation and is facilitated by the human immunodeficiency virus protease inhibitor ritonavir. Biochem Biophys Res Commun 2012;417:223–230.
13. Yin, MT, Modaressi R, Shane E, *et al.:* Effects of HIV infection and antiretroviral therapy with ritonavir on induction of osteoclast-like cells in postmenopausal women. Osteoporos Int 2011;22:1459–1468.
14. Curran A, Martinez E, Saumoy M, *et al.:* Body composition changes after switching from protease inhibitors to raltegravir: SPIRAL-LIP substudy. AIDS 2012;26:475–481.
15. Van Rompay KKA, Brignolo LL, Meyer DJ, *et al.:* Biological effects of short-term or prolonged administration of 9-[2-(phosphonomethoxy) propyl] adenine (tenofovir) to newborn and infant rhesus macaques. Antimicrob Agents Chemother 2004;48:1469–1487.
16. Kinai E and Hanabusa H: Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. AIDS Res Hum Retroviruses 2009;25:387–394.
17. Gatanaga H, Tachikawa N, Kikuchi Y, *et al.:* Urinary beta2-microglobulin as a possible sensitive marker for renal injury caused by tenofovir disoproxil fumarate. AIDS Res Hum Retroviruses 2006;22:744–748.
18. Gerstner G, Damiano ML, Tom A, *et al.:* Prevalence and risk factors associated with decreased bone mineral density in patients with haemophilia. Haemophilia 2009;15:559–565.
19. Nair AP, Jjina F, Ghosh K, Madkaikar M, Shrikhande M, and Nema M: Osteoporosis in young haemophiliacs from western India. Am J Hematol 2007;82:453–457.

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Long-term exposure to tenofovir continuously decrease renal function in HIV-1-infected patients with low body weight: results from 10 years of follow-up

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Objectives: To investigate the effect of long-term tenofovir disoproxil fumarate (TDF) use on renal function, especially in patients with low body weight who are vulnerable to TDF nephrotoxicity.

Design: A single-center, observational study in Tokyo, Japan.

Methods: We performed a 10 years cohort study of 792 HIV-1-infected patients. The effect of long-term TDF use on estimated glomerular filtration rate (eGFR) was investigated on treatment-naive patients who started TDF-containing antiretroviral therapy ($n=422$) and those who started abacavir-containing antiretroviral therapy as control ($n=370$). Three renal endpoints were examined by the logistic regression model: decrement in eGFR of higher than 10 ml/min per 1.73 m^2 relative to the baseline, more than 25% decrement in eGFR, and eGFR lower than 60 ml/min per 1.73 m^2 at least 3 months apart. The loss in eGFR was estimated using linear mixed models for repeated measures.

Results: The median weight at baseline was 63 kg. TDF use increased the risk of all three renal outcomes compared with the control group: higher than 10 ml/min per 1.73 m^2 decrement in eGFR [adjusted odds ratio (OR) = 2.1, 95% confidence interval (CI) 1.45–3.14, $P<0.001$], more than 25% decrement (adjusted OR = 2.1, 95% CI 1.50–2.90, $P<0.001$), and eGFR lower than 60 ml/min per 1.73 m^2 at least 3 months apart (adjusted OR = 3.9, 95% CI 1.62–9.36, $P=0.002$). The cumulative mean loss relative to the control after 1, 2, 3, 4, and 5 years of TDF exposure was −3.8, −3.6, −5.5, −6.6, and −10.3 ml/min per 1.73 m^2, respectively, indicating that the loss in eGFR increased over time ($P<0.001$).

Conclusion: In this cohort of patients with low body weight, TDF exposure increased the risk of renal dysfunction. Furthermore, the loss in eGFR relative to the control increased continuously up to 5 years.

AIDS 2014, **28**:1903–1910

Keywords: HIV-1, low body weight, renal dysfunction, tenofovir disoproxil fumarate, treatment-naive

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Received: 11 March 2014; revised: 12 May 2014; accepted: 14 May 2014.

DOI:10.1097/QAD.0000000000000347