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研究成果の刊行に関する一覧

Hayashida T, Gatanaga H, Takahashi Y, Negishi F, Kikuchi Y, Oka S.
Trends in early and late diagnosis of HIV-1 infections in Tokyoites from 2002 to 2010.
Int J Infect Dis. 16(3): 172-177, 2012.

Nishijima T, Gatanaga H, Komatsu H, Tsukada K, Shimbo T, Aoki T, Watanabe K, Kinai E, Honda H, Tanuma J, Yazaki H, Honda M, Teruya K, Kikuchi Y, and Oka S.
Renal function declines more in tenofovir- than abacavir-based antiretroviral therapy in low-body weight treatment-naïve patients with HIV infection.
PLoS One 7: e29977, 2012.

Takano M, Okada M, Oka S, and Wagastuma Y.
The relationship between HIV testing and CD4 counts at HIV diagnosis among newly diagnosed HIV-1 patients in Japan.
Int J STD AIDS 23: 262-266, 2012.

Sassi M, Ripamonti C, Muller NJ, Yazaki H, Kutty G, Ma L, Huber C, Gogineni E, Oka S, Goto N, Fehr T, Gianella S, Konrad R, Sing A, and Kovacs JA.
Outbreaks of *Pneumocystis* pneumonia in 2 renal transplant centers linked to a single strain of *Pneumocystis*: Implications for transmission and virulence.
Clin Infect Dis 54: 1437-1444, 2012.

Nishijima T, Tsukada K, Teruya K, Gatanaga H, Kikuchi Y, and Oka S.
Efficacy and safety of once-daily ritonavir-boosted darunavir plus abacavir/lamivudine for treatment-naïve patients: A pilot study.
AIDS (Research letter) 26: 649-651, 2012.

Nagata N, Shimbo T, Nakashima R, NiikuraR, Nishimura S, Yada T, Akiyama A, Watanabe K, Oka S, and Uemura N.
Risk Factors for Intestinal Invasive Amebiasis in Japan, 2003–2009
Emerg Infect Dis 18: 717-724, 2012.

Hamada Y, Nishijima T, Watanabe K, Komatsu H, Tsukada K, Teruya K, Gatanaga H, Kikuchi Y, and Oka S.
High Incidence of Renal Stones Among HIV-Infected Patients on Ritonavir-Boosted Atazanavir Than in Those Receiving Other Protease Inhibitor–Containing Antiretroviral Therapy.
Clin Infect Dis 55(9): 1262-1269, 2012.

Nishijima T, Komastu H, Higasa K, Takano M, Tsuchiya K, Hayashida T, Oka S, and Gatanaga H.
Single nucleotide polymorphisms in *ABCC2* associate with tenofovir-induced kidney tubular dysfunction in Japanese patients with HIV-1 infection: A pharmacogenetic study.
Clin Infect Dis 55(11): 1558-1567, 2012.

Nishijima T, Komatsu H, Teruya K, Tanuma J, Tsukada K, Gatanaga H, Kikuchi Y, and Oka S.
Once-daily darunavir/ritonavir plus abacavir/lamivudine versus tenofovir/ emtricitabine for treatment-naïve patients with baseline viral load >100,000 copies/mL.
AIDS (Research Letter) 24: e32835, 2012.

Tsuzuki T, Iwase H, Shimada M, Hirashima N, Hibino Y, Ryuge N, Saito M, Tamaki D, Kamiya A, Yokoi M, Yokomaku Y, Fujisaki S, Sugiura W, Goto H.
Clinical evaluation of peginterferon alpha plus ribavirin for patients co-infected with HIV and HCV at Nagoya Medical Center.
Nihon Shokakibyo Gakkai zasshi = The Japanese journal of gastro-enterology 109(7): 1186-1196, 2012.

Miyamoto T, Nakayama EE, Yokoyama M, Ibe S, Takehara S, Kono K, Yokomaku Y, Pizzato M, Luban J, Sugiura W, Sato H, Shioda T.
The Carboxyl-Terminus of Human Immunodeficiency Virus Type 2 Circulating Recombinant form 01_AB Capsid Protein Affects Sensitivity to Human TRIM5α.
PloS one 7(10): e47757, 2012.

Kitamura S, Ode H, Nakashima M, Imahashi M, Naganawa Y, Kurosawa T, Yokomaku Y, Yamane T, Watanabe N, Suzuki A, Sugiura W, Iwatani Y.
The APOBEC3C crystal structure and the interface for HIV-1 Vif binding
Nature structural & molecular biology 19(10): 1005-1010, 2012.

Watanabe D, Yoshino M, Yagura H, Hirota K, Yonemoto H, Bando H, Yajima K, Koizumi Y, Otera H, Tominari S, Nishida Y, Kuwahara T, Uehira T, Shirasaka T.
Increase in serum mitochondrial creatine kinase levels induced by tenofovir administration.
Infect Chemother 18(5): 675-82, 2012.

Yoshino M, Yagura H, Kushida H, Yonemoto H, Bando H, Ogawa Y, Yajima K, Kasai D, Taniguchi T, Watanabe D, Nishida Y, Kuwahara T, Uehira T, Shirasaka T.
Assessing recovery of renal function after tenofovir disoproxil fumarate discontinuation.
J Infect Chemother 18(2): 169-74, 2012.

Akahoshi T, Chikata T, Tamura Y, Gatanaga H, Oka S, Takiguchi M.
Selection and accumulation of an HIV-1 escape mutant by three types of HIV-1-specific cytotoxic T lymphocytes recognizing wild-type and/or escape mutant epitopes.
J Virol. 86(4): 1971-1981, 2012.

Hasan Z, Carlson JM, Gatanaga H, Le AQ, Brumme CJ, Oka S, Brumme ZL, Ueno T.
Minor contribution of HLA class I-associated selective pressure to the variability of HIV-1 accessory protein Vpu.
Biochem Biophys Res Commun. 421(2): 291-295, 2012.

Naruto T, Gatanaga H, Nelson G, Sakai K, Carrington M, Oka S, Takiguchi M.
HLA class I-mediated control of HIV-1 in the Japanese population, in which the protective HLA-B*57 and HLA-B*27 alleles are absent.
J Virol. 86(19): 10870-10872, 2012.

Matthews PC, Koyanagi M, Kloverpris HN, Harndahl M, Stryhn A, Akahoshi T, Gatanaga H, Oka S, Juarez Molina C, Valenzuela Ponce H, Avila Rios S, Cole D, Carlson J, Payne RP, Ogwu A, Bere A, Ndung'u T, Gounder K, Chen F, Riddell L, Luzzi G, Shapiro R, Brander C, Walker B, Sewell AK, Reyes Teran G, Heckerman D, Hunter E, Buus S, Takiguchi M, Gpulder PJ.
Differential clade-specific HLA-B*3501 association with HIV-1 disease outcome is linked to immunogenicity of a single Gag epitope.
J Virol. 86(23): 12643-12654, 2012.

Nishijima T, Yazaki H, Hinoshita F, Tasato D, Hoshimoto K, Teruya K, Gatanaga H, Kikuchi Y, Oka S.
Drug-induced acute interstitial nephritis mimicking acute tubular necrosis after initiation of tenofovir-containing antiretroviral therapy in patient with HIV-1 infection.
Intern Med. 51(17): 2469-2471, 2012.

Kinai E, Hosokawa S, Gomibuchi H, Gatanaga H, Kikuchi Y, Oka S.
Blunted fetal growth by tenofovir in late pregnancy.
AIDS. 26(16): 2119-2120, 2012.

Yagita Y, Kuse N, Kuroki K, Gatanaga H, Carlson JM, Chikata T, Brumme ZL, Murakoshi H, Akahoshi T, Pfeifer N, Mallal S, John M, Ose T, Matsubara H, Kanda R, Fukunaga Y, Honda K, Kawashima Y, Ariumi Y, Oka S, Maenaka K, Takiguchi M.
Distinct HIV-1 Escape Patterns Selected by Cytotoxic T Cells with Identical Epitope Specificity.
J Virol. 87(4): 2253-2263, 2013.

Honda H, Gatanaga H, Aoki T, Watanabe K, Yazaki H, Tanuma J, Tsukada K, Honda M, Teruya K, Kikuchi Y, Oka S.
Raltegravir can be used safely in HIV-1-infected patients treated with warfarin.
Int J STD AIDS. 23(12): 903-904, 2012.

Sudo S, Haraguchi H, Hirai Y, Gatanaga H, Sakuragi JI, Momose F, Morikawa Y.
Efavirenz enhances HIV-1 Gag processing at the plasma membrane through Gag-Pol dimerization.
J Virol. In press

Hamada Y, Nagata N, Honda H, Teruya K, Gatanaga H, Kikuchi K, Oka S.
Idiopathic oropharyngeal and esophageal ulcers related to HIV infection successfully treated with antiretroviral therapy alone.
Intern Med. 52(3): 393-395, 2013.

Bunupuradah T, Imahashi M, Iampornsin T, Matsuoka K, Iwatani Y, Puthanakit T, Ananworanich J, Sophonphan J, Mahanontharit A, Naoe T, Vonthanak S, Phanuphak P, Sugiura W.
On Behalf Of The Predict Study Team. Association of APOBEC3G genotypesand CD4 decline in Thai and Cambodian HIV-infected children with moderate immune deficiency.
AIDS Res Ther 9(1): 34, 2012.

Ode H, Nakashima M, Kitamura S, Sugiura W, Sato H.
Molecular dynamics simulation in virus research
Frontiers in microbiology 3: 258, 2012.

Matsunaga S, Sawasaki T, Ode H, Morishita R, Furukawa A, Sakuma R, Sugiura W, Sato H, Katahira M, Takaori-Kondo A, Yamamoto N, Ryo A.
Molecular and enzymatic characterization of XMRV protease by a cell-free proteolytic analysis.
Journal of proteomics 75(15): 4863-4873, 2012.

Jahanbakhsh F, Ibe S, Hattori J, Monavari SH, Matsuda M, Maejima M, Iwatani Y, Memarnejadian A, Keyvani H, Azadmanesh K, Sugiura W.
Molecular epidemiology of HIV-1 infection in Iran: genomic evidence of CRF35_AD predominance and CRF01_AE infection among individuals associated with injection drug use.
AIDS research and human retroviruses 29: 198-203, 2012.

佐藤典宏
「HIV・HCV 重複感染症診療ガイドライン　改訂第 5 版」（平成 24 年 10 月刊行）
編集：北海道大学病院 HIV・HCV 重複感染症診療委員会
概要：HIV・HCV 重複感染症診療の実際をコンパクトにまとめた。北海道大学病院の血液内科、肝臓内科、移植外科の専門医等が執筆。17 ページ。
発行：500 部

佐藤典宏
「HIV・HCV 重複感染患者さんの手引き　Heartec　改訂第 5 版」（平成 24 年 10 月刊行）
編集：北海道大学病院 HIV・HCV 重複感染症診療委員会
概要：上記ガイドラインの姉妹編で、患者用パンフレット。27 ページ。
発行：500 部

佐藤典宏
「平成 24 年度北海道 HIV/AIDS 医療者研修会記録集」（平成 24 年 12 月刊行）
編集：北海道大学病院 HIV 感染症対策委員会
概要：平成 24 年度北海道 HIV/AIDS 医療者研修会で行われた講演の記録集。5 つの講演内容を収載。
66 ページ
発行：200 部

伊藤俊広
東北における HIV 感染症の動向・現状・課題
医薬の門 52(6)：456-460、2012.

上田幹夫
北陸地方における HIV 感染の動向と現状
医薬の門 52(1)：16-20、2012.

上平朝子
【日本における HIV 感染症の動向と現状】近畿地区における HIV 感染の動向と現状.
「医薬の門」 52 巻 3 号，2012 年.

上平朝子
施設紹介　HIV チーム医療の現場から～私たちが実践している工夫と取り組み～.
「HIV BODY AND MIND」 1 巻 1 号，2012 年.

齊藤誠司，鍵浦文子，喜花伸子，舩附祥子，藤田啓子，畝井浩子，藤井輝久，高田　昇，木村昭郎
HIV/HBV 重複感染症例における HBV に対する治療経験とその考察
日本エイズ学会雑誌 14：111-117，2012.

藤井輝久
エイズ/HIV 感染症の概略と検査の勧め方－実習を通じて－
広島市医師会だより 554：7-9，2012.

藤井輝久
中国四国地方における HIV 感染症の動向と現状
医学の門 53：262-267，2012.

前田憲昭、溝部潤子、大多和由美、宮田　勝、宇佐美雄司、吉川博政、柴　秀樹、有家　巧、高木律男、北川善政、秋野憲一、池田健太郎
「HIV 感染者の歯科医療の充実に向けて」歯科医師研修資料
歯科の医療体制整備，2012 年 9 月

山中京子
パンフレット名：エイズ派遣カウンセリング制度の活用方法（本体）
配付の対象：派遣カウンセラー、派遣カウンセリング制度の行政担当者、行政のエイズ対策担当者
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発行部数：300 部
発行年：2013 年 3 月

田中千枝子
「ソーシャルワーク研究にとってエビデンスとは何か」『ソーシャルワーク学』.
日本ソーシャルワーク学会，2013 年 3 月．in press

田村光平，小川俊夫，白阪琢磨，井出博生，川﨑忠記，原野廣子，今村知明.
HIV 診療の原価計算に関する一考察
医療情報学論文集 32(suppl.)：320-323，2012.

吉野宗宏，矢倉裕輝，櫛田宏幸，米本仁史，廣田和之，板東裕基，矢嶋敬史郎，小泉祐介，大寺　博，富成伸次郎，渡邊　大，栗原　健，西田恭治，上平朝子，白阪琢磨.
当院における 1 日 1 回投与 ダルナビル/リトナビルの使用成績.
日本エイズ学会誌 14：141-145，2012.

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Trends in early and late diagnosis of HIV-1 infections in Tokyoites from 2002 to 2010

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SUMMARY

Objective: The objective of this study was to delineate the trends in early and late diagnosis of HIV-1 infection in newly diagnosed Tokyoites.
Methods: The BED assay was used to identify cases diagnosed at an early stage of infection. BED-positive non-AIDS cases with a CD4 cell count ≥200/μl were defined as cases with recent infection. The rates of AIDS and recent infection in 809 newly diagnosed Tokyoites during 2002–2010 were analyzed.
Results: The AIDS rate was 22.5%. AIDS patients were older (40.4 years) than non-AIDS patients (35.0 years), and a smaller proportion were men who have sex with men (MSM) in AIDS patients (81.7%) than in non-AIDS patients (89.9%). The AIDS rate was persistently lower (≤14.3%) in ≤29-year-old than in ≥30-year-old MSM. The rate of recent infection was 24.4%. Individuals with recent infection (33.0 years old) were younger than the others (37.2 years). The rate of recent infection was lower (≤18.5%) in MSM aged ≥40 years than in those aged ≤39 years during the study period, except for 2007 and 2008.
Conclusions: Younger MSM Tokyoites appear to be aware of the risk of their sexual behavior, sufficient to take voluntary HIV testing repeatedly, resulting in early diagnosis. Older MSM did not take HIV testing frequently enough and may be a good target for campaigns promoting testing.

1. Introduction

The overall growth of the global AIDS epidemic appears to have stabilized. The annual number of new cases of HIV infection has been in steady decline since the late 1990s.[1] In Japan, however, the annual number of newly diagnosed cases has almost doubled during the most recent decade (791 cases in 2000 and 1544 cases in 2010), although the prevalence of HIV in the adult population remains <0.1%.[2] The distribution of these cases is heavily concentrated in large cities, and approximately 35% of the newly diagnosed cases have been identified in Tokyo.[3]

Early diagnosis of HIV infection is critically important because some AIDS-defining diseases are fatal, even in the era of combination antiretroviral treatment (ART); also the introduction of ART after the development of AIDS is often complicated with immune reconstitution inflammatory syndrome (IRIS).[4,5] In this regard, the introduction of ART at the early stages seems to significantly reduce the sexual transmission of HIV-1.[6,7] Thus, it is important to identify newly infected individuals and provide early ART to reduce the incidence of AIDS and transmission of HIV. Knowledge about the proportion of patients diagnosed at the early stage of an HIV infection in the newly diagnosed cases is also useful for planning and evaluation of any prevention program and for resource allocation.[8,9] However, it is usually difficult to distinguish recent from long-standing HIV infections except for acute symptomatic infections.[10] Simple prediction of the infection time from CD4 cell counts appears inaccurate because the disease progression rate varies enormously among infected individuals.[11] The BED HIV-1 capture enzyme immunoassay (BED assay) uses the branched peptide to detect HIV-1 IgG antibodies from all subtypes (i.e., HIV-1 B, E, and D gp41 immunodominant sequences are included on a branched peptide used in the assay) and measures levels of anti-HIV-1 IgG relative to total IgG.[12] Since the ratio of anti-HIV-1 IgG to total IgG increases with time shortly after HIV-1 infection, the HIV-1-infected patient is considered to have recently acquired the infection when the normalized optical density (ODn) is less than 0.8 on the BED assay (ODn reaches 0.8 on average 197 days after seroconversion.).[13]

The present study was an attempt to delineate the trends in early diagnosis of HIV-1 infection in Tokyo from 2002 to 2010 by using the BED assay. The aim of this analysis was to enhance our understanding of the status of HIV-1 spread in Tokyo and to help in the design of strategies to control the HIV-1 epidemic in Japan.

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2. Materials and methods

2.1. Newly diagnosed patients

This study included all ART-naïve HIV-1-infected individuals who met the following criteria: (1) those who visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, between 2002 and 2010 within 30 days of their diagnosis with an HIV-1 infection and (2) availability of plasma samples taken at the first visit under signed informed consent for use in viral, immunological, and epidemiological studies. Participant information including CD4 count, HIV-1 load, age at the first visit, gender, nationality, probable HIV-1 transmission route, and history of HIV testing, were collected from the medical records. According to the Japanese law for infection control, physicians are obliged to report newly diagnosed HIV/AIDS cases to the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government). A total of 11 673 HIV/AIDS cases nationally, including 4048 cases diagnosed in Tokyo (Tokyo cases), which were entered into the registry of this committee from 2002 to 2010, were used as the control populations to evaluate the representativeness of the patients enrolled in the present study (AIDS Clinical Center cases).[2,3] Plasma samples obtained from the participants were stored at −80 °C. The viral subtype in each case was determined from the HIV-1 protease–reverse transcriptase sequence (which was analyzed for drug resistance genotyping) by the neighbor-joining method using the Genetic-Win system (Software Development, Tokyo).[14]

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the ethics committee of the National Center for Global Health and Medicine.

2.2. BED assay

The BED HIV-1 capture enzyme immunoassay (BED assay; Calypte Biomedical Corp., Portland, OR, USA) was used to estimate the time of HIV-1 infection.[12] In accordance with the manufacturer's instructions, 5 μl of plasma was diluted with 500 μl of the diluent in the kit, and the proportion of anti-HIV-1-specific IgG to the total IgG in the sample was measured by optical density (OD). The OD values of the test specimens were normalized (ODn) relative to the value of a calibrator (specimen OD/calibrator OD) to minimize inter-run variation. Samples with ODn ≤0.8 were considered to be from individuals who had seroconverted within 197 days and were defined as BED-positive.[13] BED-positive non-AIDS cases with CD4 cell counts ≥200/μl were defined as individuals with recent infection. The others were defined as chronic infection.

2.3. Statistical analysis

Differences in demographic data including age, gender, risk behavior, nationality, and AIDS development among the AIDS Clinical Center cases, national cases, and Tokyo cases, were examined for significance using one-way analysis of variance (ANOVA) and the Tukey test, or Pearson's Chi-square test. Differences in demographic data including age, CD4 count, logarithmic HIV-1 viral load, nationality, transmission category, HIV-1 subtype, cue for HIV diagnosis, and history of HIV testing, between AIDS and non-AIDS patients and between recent and chronic infection, were examined for significance using the *t*-test or Pearson's Chi-square test. To estimate the correlation with the development of AIDS, binominal logistic regression analysis including age, nationality (Japanese or not), and transmission category (men having sex with men (MSM) or not) was performed. A *p*-value of less than 5% denoted statistical significance. Statistical analyses were performed with SPSS Statistics 17.0 (IBM Japan Inc., Tokyo, Japan) and Stat Mate II (NANKODO, Tokyo).

3. Results

3.1. Newly diagnosed cases of HIV-1 infection

The study subjects were 809 ART-naïve HIV-1-infected patients. All of them had visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, within 30 days of the diagnosis of HIV-1 infection (median 8 days) between 2002 and 2010. They included 741 Japanese, 35 Asians other than Japanese, and 33 from other countries. They represented 20.0% of the total number of newly diagnosed Tokyoite cases during the same period (Table 1). There were no significant differences in the proportion of AIDS (22.5% vs. 21.9%), percentage of males (96.2% vs. 94.3%), or proportion of Japanese (91.6% vs. 90.7%) between our study patients and those of the Tokyo registry, although our patients included a significantly smaller proportion of AIDS cases (22.5% vs. 30.4%) and significantly larger population of male patients (96.2% vs. 91.8%) and Japanese patients (91.6% vs. 88.5%) compared with the patients of the national registry. Furthermore, our patients were significantly younger than the patients of the Tokyo and national registries (36.2 vs. 37.7 and 38.0 years), and the proportion of MSM among male patients was significantly higher than in the Tokyo and national registries (88.0% vs. 72.8% and 59.8%).

Subtype analysis successfully determined the HIV-1 subtype in 807 patients (99.8%); the majority were infected with HIV-1 subtype B (742 patients, 91.9%), while 5.7% were infected with HIV-1 subtype AE, which is comparable to previously published subtype data in Japan.[14] The HIV-1 subtype could not be determined in two patients because the viral load was below the detection limit (<40 copies/ml), although they were not being treated with anti-HIV drugs.

3.2. Features of AIDS patients

Among the 809 cases, 182 (22.5%, 95% confidence interval (95% CI) 19.6–25.4) had already developed AIDS at the first visit, while the other 627 were non-AIDS cases (Table 2). AIDS cases were significantly older (40.4 years, 95% CI 38.8–41.9 vs. 35.0 years, 95% CI 34.2–35.9), and as expected, had lower CD4 counts (61.7/μl, 95% CI 50.6–72.8 vs. 318.0/μl, 95% CI 303.0–333.0) and higher viral loads (5.22 log VL/ml, 95% CI 5.13–5.31 vs. 4.63 log VL/ml, 95% CI 4.56–4.70) than non-AIDS patients. There were no significant differences in nationality (Japanese 91.8%, 95% CI 87.8–95.8 vs. 91.5%, 95% CI 89.4–93.7) or HIV-1 subtype (subtype B 89.0%, 95% CI 84.5–93.6 vs. 92.5%, 95% CI 90.4–94.6) between AIDS and non-AIDS

Table 1
New cases of HIV-1-infected patients diagnosed between 2002 and 2010

	Japan[a]	Tokyo[b]	This study
Number of cases	11 673	4048	809
Age, years (mean ± SD)	38.0 ± 11.8[c]	37.7 ± 11.9[d]	36.2 ± 11.0
Males	10 721 (91.8%)[c]	3819 (94.3%)	778 (96.2%)
Men having sex with men	6408 (59.8%)[c]	2780 (72.8%)[c]	685 (88.0%)
Japanese	10 335 (88.5%)[d]	3673 (90.7%)	741 (91.6%)
AIDS cases	3551 (30.4%)[c]	885 (21.9%)	182 (22.5%)

Statistical analyses were performed by one-way ANOVA and Tukey test, or Chi-square test.
[a] Provided by the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government).
[b] Provided by the Bureau of Social Welfare and Public Health, Tokyo.
[c] $p < 0.001$, compared with the study participants.
[d] $p < 0.01$ compared with the study participants.

Table 2
Demographics of participants with and without AIDS

	AIDS (n=182)		Non-AIDS (n=627)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	40.4	(38.8–41.9)	35.0	(34.2–35.9)	<0.001
CD4 count /μl	61.7	(50.6–72.8)	318.0	(303.0–333.0)	<0.001
Log viral load/ml	5.22	(5.13–5.31)	4.63	(4.56–4.70)	<0.001
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.424
Japan	167	91.8 (87.8–95.8)	574	91.5 (89.4–93.7)	
Asia other than Japan	11	6.0 (3.3–10.8)	24	3.8 (2.6–5.7)	
North and South America	2	1.1 (0.2–4.0)	17	2.7 (1.7–4.3)	
Africa	2	1.1 (0.2–4.0)	6	1.0 (0.4–2.1)	
East and West Europe	0	0 (0–2.0)	4	0.6 (0.2–1.6)	
Oceania	0	0 (0–2.0)	2	0.3 (0–1.1)	
Transmission category					
Male	175	96.2 (93.4–98.9)	603	96.2 (94.7–97.7)	0.024
MSM	143	81.7 (76.0–87.4)	542	89.9 (87.5–92.3)	
Heterosexual	21	12.0 (7.2–16.8)	43	7.1 (5.4–9.6)	
IDU	1	0.6 (0–3.2)	2	0.3 (0.1–1.2)	
Unknown	10	5.7 (3.0–10.5)	16	2.7 (1.6–4.3)	
Female	7	3.8 (1.7–7.9)	24	3.8 (2.6–5.7)	
Heterosexual	7	100 (46.8–100)	24	100 (100–100)	
Subtype					0.351
B	162	89.0 (84.5–93.6)	580	92.5 (90.4–94.6)	
AE	16	8.8 (5.4–14.3)	30	4.8 (3.4–6.8)	
C	1	0.5 (0–3.0)	7	1.1 (0.5–2.3)	
G	2	1.1 (0.2–4.0)	3	0.5 (0.1–1.4)	
AG	1	0.5 (0–3.0)	3	0.5 (0.1–1.4)	
A	0	0 (0–2.0)	2	0.3 (0–1.1)	
Unknown	0	0 (0–2.0)	2	0.3 (0–1.1)	
Cue for HIV diagnosis					<0.001
Voluntary testing	12	6.6 (3.7–11.5)	283	45.1 (41.2–49.0)	
Provider-initiated testing	167	91.8 (87.8–95.8)	338	53.9 (50.0–57.8)	
Unknown	3	1.6 (0.4–4.8)	6	1.0 (0.4–2.1)	
Previous testing					<0.001
Yes	29	15.9 (10.6–21.3)	282	45.0 (41.1–48.9)	
No	65	35.7 (28.8–42.7)	254	40.5 (36.7–44.4)	
Unknown	88	48.4 (41.1–55.6)	91	14.5 (11.8–17.3)	
BED assay					<0.001
Recent (ODn ≤0.8)	47	25.8 (19.5–32.2)	255	40.7 (36.8–44.5)	
Chronic (ODn >0.8)	135	74.2 (67.8–80.5)	372	59.3 (55.5–63.2)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user; ODn, normalized optical density.
[a] By t-test or Pearson's Chi-square test.

cases (Pearson's Chi-square test). MSM activity was the most frequent transmission route in both groups, and still more frequent in non-AIDS cases (89.9%, 95% CI 87.5–92.3) than in AIDS cases (81.7%, 95% CI 76.0–87.4). A larger proportion of patients in the non-AIDS group than in the AIDS group had undertaken previous HIV testing (45.0%, 95% CI 41.1–48.9 vs. 15.9%, 95% CI 10.6–21.3) and had been diagnosed with HIV-1 infection by voluntary testing (45.1%, 95% CI 41.2–49.0 vs. 6.6%, 95% CI 3.7–11.5), suggesting that repeated voluntary testing may prevent disease progression to AIDS in the high-risk groups.

Binominal logistic regression analysis of age, nationality (Japanese or not), and transmission category (MSM or not) identified age as the most significant factor associated with the development of AIDS (per 1-year increment, (hazard ratio) HR 1.041, 95% CI 1.026–1.057; $p < 0.001$).

To delineate the trends in late diagnosis of HIV-1 infection, the annual rates of AIDS cases in newly-diagnosed HIV-1-infected patients were plotted through the study period. The rate of AIDS cases remained around 30% between 2002 and 2004. It decreased to 15.0% in 2005, but then showed a gradual increase annually, reaching 24.8% in 2010 (Figure 1). To identify the population that influenced the increase in the rate of AIDS cases in the most recent years, we selected and categorized the study participants based on their features. Specifically, we focused on MSM patients, because 85% of our patients were MSM. Based on the above results of the significance of age in the binominal logistic regression analysis in the development of AIDS, we examined the effect of age in more detail by dividing the MSM patients into three age groups: those aged ≤29 years (217 patients, 31.7%), 30–39 years (273 patients, 39.9%), and ≥40 years (195 patients, 28.5%). In the ≥40 years MSM group, the rate was higher than 50% between 2002 and 2004, but decreased to 21.4% in 2005 and further decreased to 14.3% in 2006, but gradually increased and reached ~30% in 2009 and 2010 (Figure 1). On the other hand, in the ≤29 years MSM group, the AIDS rate was steadily lower than 20%, indicating that most young HIV-1-infected MSM were diagnosed before the development of AIDS throughout the study period. The AIDS rate in the 30–39 years MSM group was between those of the other two groups during most of the study period. A significantly larger proportion of patients in the ≤29 years MSM group had undergone voluntary HIV testing (43.8%, $p = 0.002$, Pearson's Chi-square test) and diagnosis with HIV (48.8%, $p < 0.001$, Pearson's Chi-square test), compared with the 30–39 years MSM group (43.6% and 36.6%, respectively) and the ≥40 years MSM group (34.9% and 32.3%, respectively). These results suggest that repeated voluntary testing may have prevented disease progression to AIDS in the younger MSM groups. The high rate of AIDS in all the study participants observed in 2002–2004 seemed mainly due to the ≥40-year-old MSM. Furthermore, the gradual increase in the AIDS rate in the ≥40-year-old MSM since 2006 also seemed to have contributed to

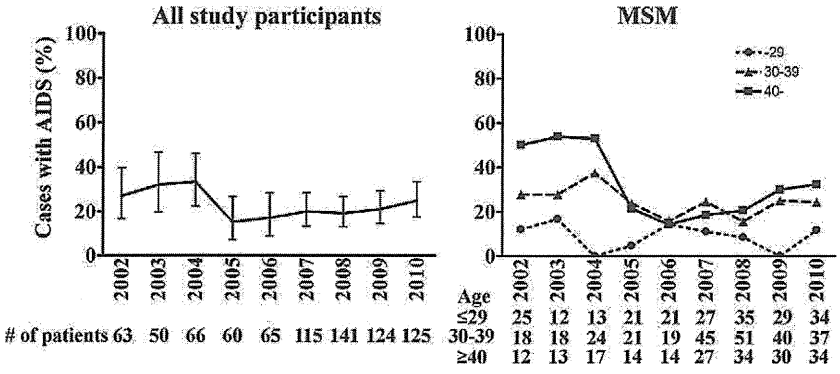

Figure 1. Annual rate of AIDS in newly diagnosed HIV-1-infected individuals. The annual AIDS rate for all study participants (809 patients; left panel), and men who have sex with men (MSM) categorized by age: ≤29 years (n = 217), 30–39 years (n = 273), and ≥40 years (n = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S1).

the rising AIDS rate in all, suggesting that older MSM should be the main target for interventions aimed at promoting HIV testing for early diagnosis and prevention of the development of AIDS.

3.3. Trends in early HIV diagnosis

To identify individuals with recent HIV-1 infection, we performed a BED assay for the 809 study participants. Before analysis of the results, we dealt with the problem of potential misclassification. Previous studies reported small levels of anti-HIV-1-specific IgG relative to the total IgG in cases with both recent HIV-1 infection and long-standing chronic cases with severe immunodeficiency, which could result in false classification of chronic cases as recent infection.[12,15,16] To tackle this problem, previous studies classified AIDS cases and cases with CD4 cell counts <200/μl as chronic infection cases, in accordance with the Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) guidelines.[17–21] We applied

Table 3
Demographics of participants with recent and chronic infection

	Recent (n = 197)		Chronic (n = 612)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	33.0	(31.7–34.3)	37.2	(36.3–38.1)	<0.001
CD4 count /μl	423.2	(399.2–447.3)	207.9	(193.3–222.4)	<0.001
Log viral load/ml	4.61	(4.46–4.76)	4.81	(4.74–4.87)	0.005
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.101
Japan	189	95.9 (93.2–98.7)	552	90.2 (87.8–92.6)	
Asia other than Japan	2	1.0 (0.2–3.7)	33	5.4 (3.9–7.6)	
North and South America	3	1.5 (0.4–4.4)	16	2.6 (1.6–4.2)	
Africa	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
East and West Europe	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
Oceania	1	0.5 (0–2.8)	1	0.2 (0–0.9)	
Transmission category					
Male	192	97.5 (95.3–99.7)	586	95.8 (94.2–97.3)	0.314
MSM	177	92.2 (88.4–96.0)	508	86.7 (83.9–89.4)	
Heterosexual	11	5.7 (3.1–10.2)	53	9.0 (7.0–11.8)	
IDU	0	0 (0–1.9)	3	0.5 (0.1–1.5)	
Unknown	4	2.1 (0.7–5.3)	22	3.8 (2.5–5.7)	
Female	5	2.5 (1.0–5.9)	26	4.2 (2.9–6.2)	-
Heterosexual	5	100 (34.4–100)	26	100 (81.5–100)	
Subtype					0.029
B	188	95.4 (92.5–98.3)	554	90.5 (88.2–92.8)	
AE	4	2.0 (0.7–5.2)	42	6.9 (5.2–9.3)	
C	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
G	1	0.5 (0–2.8)	4	0.7 (0.2–1.7)	
AG	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
A	0	0 (0–1.9)	2	0.3 (0–1.2)	
Unknown	2	1.0 (0.2–3.7)	0	0 (0–0.6)	
Cue for HIV diagnosis					<0.001
Voluntary testing	102	51.8 (44.8–58.8)	193	31.5 (27.9–35.2)	
Provider-initiated testing	94	47.7 (40.7–54.7)	411	67.2 (63.4–70.9)	
Unknown	1	0.5 (0–2.8)	8	1.3 (0.6–2.6)	
Previous testing					<0.001
Yes	116	58.9 (52.0–65.8)	195	31.9 (28.2–35.6)	
No	57	28.9 (22.6–35.3)	262	42.8 (38.9–46.7)	
Unknown	24	12.2 (7.6–16.8)	155	25.3 (21.9–28.8)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user.
[a] By t-test or Pearson's Chi-square test.

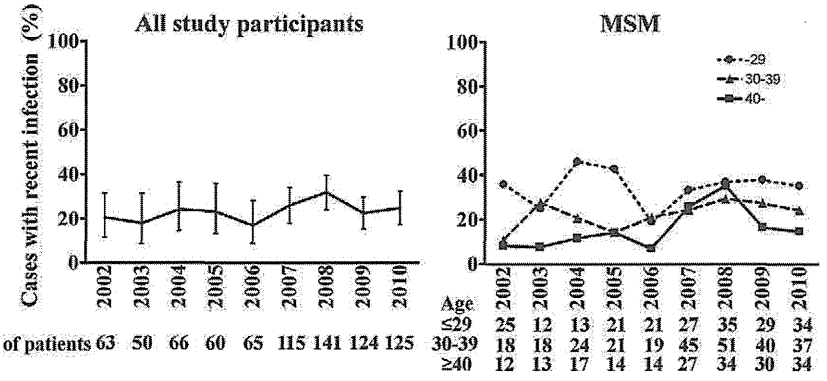

Figure 2. Annual rate of recent infection in newly diagnosed HIV-1-infected cases. The annual rate of recent infection in all study participants (809 patients; left panel), and in men who have sex with men (MSM) categorized by age: ≤29 years (n = 217), 30–39 years (n = 273), and ≥40 years (n = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S2).

the same strategy in this study and thus defined only BED-positive non-AIDS cases with CD4 cell counts ≥200/μl as recent infection.

In the 456 non-AIDS cases with CD4 cell counts ≥200/μl, 197 cases were BED-positive and classified as recent infection (43.2%; 24.4% of the total cases) (Table 3). BED-negative cases, AIDS cases, and cases with CD4 cell counts <200/μl were classified as chronic infection. Patients with recent infection were younger (33.0 years, 95% CI 31.7–34.3 vs. 37.2 years, 95% CI 36.3–38.1) and had higher CD4 counts (423.2/μl, 95% CI 399.2–447.3 vs. 207.9/μl, 95% CI 193.3–222.4), as expected, and lower viral load (4.61 log VL/ml, 95% CI 4.46–4.76 vs. 4.81 log VL/ml, 95% CI 4.74–4.87), compared to patients with chronic infection. A larger proportion of recent infection (95.4%, 95% CI 92.5–98.3) was caused by HIV-1 subtype B than in those with chronic infection (90.5%, 95% CI 88.2–92.8). There were no significant differences in the nationality and transmission category between recent and chronic infection cases (Pearson's Chi-square test), although the proportion of Japanese patients was higher in recent infection (95.9%, 95% CI 93.2–98.7) than in chronic infection (90.2%, 95% CI 87.8–92.6) (p = 0.012, Chi-square test). A significantly larger proportion of patients underwent previous HIV testing (58.9%, 95% CI 52.0–65.8 vs. 31.9%, 95% CI 28.2–35.6) and were diagnosed with HIV-1 infection by voluntary testing (51.8%, 95% CI 44.8–58.8 vs. 31.5%, 95% CI 27.9–35.2) among recent infection cases than chronic infection cases (p < 0.001 in both, Pearson's Chi-square test).

To delineate the trends in early diagnosis of HIV-1 infection, the annual rate of recent infection in all 809 study participants was plotted over the study period (Figure 2). The rate was stable at ~20% between 2002 and 2010, except for 2007 (26.1%) and 2008 (31.9%), when a slight increase was evident. In order to identify the population that influenced the annual trends of early diagnosis, we focused on MSM patients and again divided them into three age groups: ≤29 years, 30–39 years, and ≥40 years. The rates of recent infection in the ≤29 and ≥40 years MSM groups were the highest and the lowest, respectively, in most years of the study period. The rate in the ≤29 years MSM group was high, ranging from 25.0% to 46.2% between 2002 and 2005, but it decreased to 19.0% in 2006, and increased again in 2007 and remained around 35% between 2007 and 2010. The rate of recent infection in the ≥40-year-old MSM group was steadily low at ~10% between 2002 and 2006, but increased in 2007 to 25.9% and 2008 to 35.3%, then decreased to around 15% in 2009 and 2010. The rate in the 30–39-year-old MSM ranged between those of the other two groups during most part of the study period. These results suggest that younger MSM tend to be diagnosed persistently earlier, whereas older MSM are usually diagnosed at a later stage of the HIV disease.

4. Discussion

The present study analyzed the trends in the proportion of AIDS patients and patients with recent infection among 809 new cases of HIV-1-infection diagnosed between 2002 and 2010. This group recruited from our AIDS Clinical Center represents 20.0% of the total number of newly diagnosed Tokyoites during the same period. We found that MSM, especially younger MSM, tend to be diagnosed at an earlier stage before the development of AIDS, probably because of frequent voluntary HIV testing. The proportion of AIDS cases remained at a steady low level and the rate of recent infection remained at a high level in younger MSM patients, indicating that younger MSM are aware of the risk of their sexual behavior sufficient to take HIV testing repeatedly. On the other hand, in the older MSM, the rate of AIDS was relatively high and the rate of recent infection comparatively low, but transiently increased in 2007 and 2008, suggesting that older MSM with a high-risk of HIV infection usually do not take HIV testing frequently and may respond to campaigns that promote such tests. Interestingly, the Japan Foundation for AIDS Prevention conducted several campaigns to promote voluntary HIV-1 testing in 2007. A popular male Japanese singer took part in one such campaign in July 2007, which was a great surprise among the Japanese in general, and this was followed by an increase in the number of voluntary HIV tests performed in 2007 and 2008.[2] The event may have prompted older MSM at high risk to take voluntary HIV testing, resulting in the transient increase in the rate of early diagnosis for 2007 and 2008. The sharp decline in the rate of early diagnosis observed in 2009 and 2010 in the older MSM group coincided with reductions in the number of voluntary tests,[2] and could be an omen of future increases in the number of AIDS patients in this population. Early diagnosis followed by early introduction of ART may reduce the spread of HIV-1 among MSM, which could help to prevent an HIV epidemic in this population.[6,7,22] A strategy based on the promotion of voluntary testing needs to be formulated, similar to the 2007 campaigns that resulted in significant increases in the rate of early diagnosis in older MSM.

Discordant shifts were observed between the rates of AIDS and recent infection. The reasons may be that AIDS usually develops several years after HIV infection and that disease progression varies enormously among infected individuals. Therefore, the variable length of time during which HIV infection was ignored resulted in the development of AIDS, the proportion of which does not always correlate with the rate of recent infection in the same year.[11] Furthermore, disease progression has been suggested to have become faster in a significant portion of Japanese patients, probably because the prevailing HIV-1 strains in Japan have

adapted to the Japanese population by acquiring escape mutations from immune pressure restricted by human leukocyte antigens (HLAs) popular among the Japanese.[23,24] Based on this point of view, early diagnosis is even more important due to the shorter asymptomatic period before the development of AIDS.

The majority of our study participants were infected with HIV-1 subtype B, and HIV-1 subtype B infection correlated significantly with MSM (crude odds ratio 37.9, $p < 0.001$; Chi-square test). The non-AIDS patients were more likely to be infected with subtype B than AIDS patients (crude odds ratio 1.59, $p = 0.098$). The same was true for recent infection than chronic infection (crude odds ratio 2.81, $p = 0.009$). A previous Japan-wide survey also showed a close relationship between subtype B and MSM in Japan; all cases diagnosed with primary HIV-1 infection ($n = 45$) were caused by subtype B, and such primary infections were significantly frequent among MSM.[14] Considered together, the results indicate that subtype B is the major currently prevalent strain in Japan, especially among MSM, and such strains are probably adapting to the Japanese population by repeated exposure to immune pressure of the Japanese.

This study used case reporting-based surveillance to estimate the number of new HIV-1 infections in Tokyoites between 2002 and 2010. The data were collected at a single center and thus may have included some institutional bias. The study participants were statistically younger and were more likely to be MSM than those of the Tokyo registry. The BED assay was used in this study to determine the rate of recent infection in the selection study group and not to determine the national incidence rate. However, the data from this study suggest the following target-specific differential strategies for controlling the HIV epidemic and for AIDS prevention in Tokyo: campaigns aimed at promoting testing should be directed at older MSM for early diagnosis to prevent/halt the progression of AIDS; commencement of ART for HIV-infected younger MSM at early stages of the disease may effectively reduce the number of new cases based on the control of current hot-spots of HIV transmission among this group.

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Conflict of interest: The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2011.11.003.

References

1. Global report: UNAIDS report on the global AIDS epidemic 2010. UNAIDS; 2010. Available at: http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf (accessed on 2 November 2011).
2. AIDS Prevention Information Network (API-Net). Comment of National AIDS Surveillance Committee, Japan, 2011. API-Net. Available at: http://api-net.jfap.or.jp/status/index.html (accessed on 2 November 2011).
3. Bureau of Social Welfare and Public Health, Tokyo. AIDS Newsletter No. 135, 2011. Bureau of Social Welfare and Public Health. Available at: http://www.fukushihoken.metro.tokyo.jp/iryo/kansen/aids/newsletter/files/AIDS_News_Letter_No.135-1.pdf (accessed on 2 November 2011).
4. Müller M, Wandel S, Colebunders R, Attia S, Furrer H, Egger M, et al. Immune reconstitution inflammatory syndrome in patients starting antiretroviral therapy for HIV infection: a systemic review and meta-analysis. *Lancet Infect Dis* 2010;**10**:251–61.
5. Lipman M, Breen R. Immune reconstitution inflammatory syndrome in HIV. *Curr Opin Infect Dis* 2006;**19**:20–5.
6. Donnell D, Baeten JM, Kiarie J, Thomas KK, Stevens W, Cohen CR, et al. Heterosexual HIV-1 transmission after initiation of antiretroviral therapy: a prospective cohort analysis. *Lancet* 2010;**375**:2092–8.
7. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011;**365**:493–505.
8. Murillo W, Paz-Bailey G, Morales S, Monterroso E, Paredes M, Dobbs T, et al. Transmitted drug resistance and type of infection in newly diagnosed HIV-1 individuals in Honduras. *J Clin Virol* 2010;**49**:239–44.
9. Kao CF, Chang SY, Hsia KT, Chang FY, Yang CH, Liu HR, et al. Surveillance of HIV type 1 recent infection and molecular epidemiology among different risk behaviors between 2007 and 2009 after the HIV type 1 CRF07_BC outbreak in Taiwan. *AIDS Res Hum Retroviruses* 2011;**27**:745–9.
10. Cohen MS, Gay CL, Busch MP, Hecht FM. The detection of acute HIV infection. *J Infect Dis* 2010;**202**:S270–7.
11. Casado C, Colombo S, Rauch A, Martinez R, Gunthard HF, Garcia S, et al. Host and viral genetic correlates of clinical definitions of HIV-1 disease progression. *PLoS One* 2010;**5**:11079.
12. Parekh BS, Kennedy MS, Dobbs T, Pau CP, Byers R, Green T, et al. Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. *AIDS Res Hum Retroviruses* 2002;**18**:295–307.
13. Parekh BS, Hanson DL, Hargrove J, Branson B, Green T, Dobbs T, et al. Determination of mean recency period for estimation of HIV type 1 incidence with the BED-capture EIA in persons infected with diverse subtypes. *AIDS Res Hum Retroviruses* 2011;**27**:265–73.
14. Gatanaga H, Ibe S, Matsuda M, Yoshida S, Asagi T, Kondo M, et al. Drug-resistant HIV-1 prevalence in patients newly diagnosed with HIV/AIDS in Japan. *Antiviral Res* 2007;**75**:75–82.
15. Le Vu S, Pillonel J, Semaille C, Bernillon P, Le Strat Y, Meyer L, et al. Principles and uses of HIV incidence estimation from recent infection testing—a review. *Euro Surveill* 2008;**13**:11–6.
16. Marinda ET, Hargrove J, Preiser W, Slabbert H, van Zyl G, Levin J, et al. Significantly diminished long-term specificity of the BED capture enzyme immunoassay among patients with HIV-1 with very low CD4 counts and those on antiretroviral therapy. *J Acquir Immune Defic Syndr* 2010;**53**:496–9.
17. Jiang Y, Wang M, Ni M, Duan S, Wang Y, Feng J, et al. HIV-1 incidence estimates using IgG-capture BED-enzyme immunoassay from surveillance sites of injection drug users in three cities of China. *AIDS* 2007;**21**:S47–51.
18. Hall HI, Song R, Rhodes P, Prejean J, An Q, Lee LM, et al. Estimation of HIV incidence in the United States. *JAMA* 2008;**300**:520–9.
19. Scheer S, Chin CS, Buckman A, McFarland W. Estimation of HIV incidence in San Francisco. *AIDS* 2009;**23**:533–4.
20. Duan S, Shen S, Bulterys M, Jia Y, Yang Y, Xiang L, et al. Estimation of HIV-1 incidence among five focal populations in Dehong, Yunnan: a hard hit area along a major drug trafficking route. *BMC Public Health* 2010;**10**:180.
21. UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance. When and how to use assays for recent infection to estimate HIV incidence at a population level. Geneva: WHO; 2011. Available at: http://www.who.int/hiv/pub/surveillance/sti_surveillance/en/index.html (accessed on 2 November 2011).
22. Fisher M, Pao D, Brown AE, Sudarshi D, Gill ON, Cane P, et al. Determinants of HIV-1 transmission in men who have sex with men: a combined clinical, epidemiological and phylogenetic approach. *AIDS* 2010;**24**:1739–47.
23. Nakamura H, Teruya K, Takano M, Tsukada K, Tanuma J, Yazaki H, et al. Clinical symptoms and courses of primary HIV-1 infection in recent years in Japan. *Intern Med* 2011;**50**:95–101.
24. Kawashima Y, Pfafferott K, Frater J, Matthews P, Payne R, Addo M, et al. Adaptation of HIV-1 to human leukocyte antigen class I. *Nature* 2009;**458**:641–5.

Renal Function Declines More in Tenofovir- than Abacavir-Based Antiretroviral Therapy in Low-Body Weight Treatment-Naïve Patients with HIV Infection

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Abstract

Objective: To compare the rate of decline of renal function in tenofovir- and abacavir-based antiretroviral therapy (ART) in low-body weight treatment-naïve patients with HIV infection.

Design: We conducted a single-center retrospective cohort study of 503 Japanese patients who commenced on either tenofovir- or abacavir-based initial ART.

Methods: The incidence of renal dysfunction, defined as more than 25% fall in estimated glomerular filtration rate (eGFR) from the baseline, was determined in each group. The effect of tenofovir on renal dysfunction was estimated by univariate and multivariate Cox hazards models as the primary exposure. Changes in eGFR until 96 weeks were estimated in both groups with a repeated measures mixed model.

Results: The median body weight of the cohort was 64 kg. The estimated incidence of renal dysfunction in the tenofovir and the abacavir arm was 9.84 per 100 and 4.55 per 100 person-years, respectively. Tenofovir was significantly associated with renal dysfunction by univariate and multivariate analysis (HR = 1.747; 95% CI, 1.152–2.648; p = 0.009) (adjusted HR = 2.080; 95% CI, 1.339–3.232; p<0.001). In subgroup analysis of the patients stratified by intertertile baseline body weight, the effect of tenofovir on renal dysfunction was more evident in patients with lower baseline body weight by multivariate analysis (≤60 kg: adjusted HR = 2.771; 95%CI, 1.494–5.139; p = 0.001) (61–68 kg: adjusted HR = 1.908; 95%CI, 0.764–4.768; p = 0.167) (>68 kg: adjusted HR = 0.997; 95%CI, 0.318–3.121; p = 0.995). The fall in eGFR was significantly greater in the tenofovir arm than the abacavir arm after starting ART (p = 0.003).

Conclusion: The incidence of renal dysfunction in low body weight patients treated with tenofovir was twice as high as those treated with abacavir. Close monitoring of renal function is recommended for patients with small body weight especially those with baseline body weight <60 kg treated with tenofovir.

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Introduction

Tenofovir disoproxil fumarate (TDF) and abacavir sulfate (ABC) are widely used nucleot(s)ide reverse transcriptase inhibitors (NRTIs) as part of the initial antiretroviral therapy for patients with HIV infection in the developed countries (URL:http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf) (URL: http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf). TDF is generally preferred to ABC, since ABC is reported to cause serious hypersensitivity reaction in 5–8% of the patients and its efficacy in viral suppression is reported to be inferior to TDF among patients with baseline HIV viral load of >100,000 copies/ml [1,2]. On the other hand, renal proximal tubular damage and renal dysfunction are well-known adverse effects of TDF [3–9]. A meta-analysis study that compared TDF and other NRTIs concluded that the decline in renal function with TDF use is significant but modest, and the ASSERT study conducted in Europe compared randomly-selected treatment naïve patients who commenced treatment with either TDF or ABC with efavirenz and showed no difference in estimated glomerular filtration

rate (eGFR) between the two groups at 48 weeks [9,10]. To date, the nephrotoxicity of TDF have been regarded as mild and tolerable [2,5–7,9–11].

However, the TDF-related nephrotoxicity has hardly been evaluated in patients with small body weight, who are potentially at higher risk for larger drug exposure and thus, more severe toxicity [12–15]. Indeed, some recent studies including ours reported a higher incidence of TDF-related renal dysfunction among Asian patients with low body weight compared with previous studies on mostly Whites and African Americans with larger body weight [13,16]. Thus, it is important to provide more evidence in support of TDF-associated nephrotoxicity in patients with low body weight since such data can elucidate whether TDF-related nephrotoxicity is as mild in low-body-weighted patients as previously reported in Europe and the USA. This is also important because there is increasing use of TDF in resource-limited settings, where patients are often of relatively small body weight, following the revised 2010 WHO guidelines that recommend TDF as one of the components of first line therapies (URL:http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf)[13,16–19]. To our knowledge, there are no studies that compared renal function in treatment naïve Asian patients who commenced treatment with TDF or ABC.

Based on the above background, the present study was designed to compare the incidence of renal dysfunction and change in eGFR between treatment-naïve Japanese patients with low body weight who started either TDF or ABC as part of the antiretroviral regimen.

Methods

Ethics Statement

This study was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine, Tokyo. All patients included in this study provided a written informed consent for their clinical and laboratory data to be used and published for research purposes. This study has been conducted according to the principles expressed in the Declaration of Helsinki.

Study Subjects

We performed a retrospective, single-center cohort study of HIV-infected Japanese patients using the medical records at the National Center for Global Health and Medicine, Tokyo, Japan. Our facility is one of the largest clinics for patients with HIV infection in Japan with more than 2,700 registered patients. The study population was treatment-naïve patients with HIV infection, aged >17 years, who commenced treatment with either the recommended 300 mg/day dose of TDF or 600 mg/day dose of ABC-containing antiretroviral regimen at our clinic between January 1, 2004 and March 31, 2009. During this inclusion period, all except two patients at our clinic started ART with either ABC or TDF. Patients with an eGFR of >60 ml/min/1.73 m^2 were enrolled. Patients were followed up until March 31, 2011. They were excluded if they started ART with both TDF and ABC, their follow-up period at our facility was less than 24 weeks after commencement of ART, or if they had started ART at other facilities. Only Japanese patients were included in order to examine a population with comparatively homogenous basic demographics and background. The attending physician selected either TDF or ABC at baseline, and the use of these two drugs was based on the Japanese guidelines, which place both ABC and TDF as the preferred NRTIs (http://www.haart-support.jp/guideline2011.pdf. in Japanese). The attending physician also selected the key drug [non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), or integrase inhibitor (INI)]. All patients received standard ART with two NRTIs combined with either PI, NNRTI, or INI.

Measurements

We defined renal dysfunction as more than 25% decrease in eGFR relative to the baseline [13,16,20,21]. The baseline eGFR was estimated for each patient from the average of two successive serum creatinine measurements made closest to and preceding the commencement of antiretroviral therapy by no more than 90 days. Changes in eGFR were plotted from the baseline measurement until the average value of two successive measurements diminished to less than 75% of the baseline, discontinuation of TDF or ABC, or at the end of the follow-up period. Discontinuation of TDF and ABC was the choice of the attending physician, and was based on virologic failure or ART-related side effects other than renal dysfunction. Before the initiation of ART and until suppression of HIV-1 viral load, patients visited our clinic every month. However, after viral load suppression, the visit interval was extended up to every three months. Serum creatinine and eGFR were measured in every visit, and the frequency of measurements was similar in patients on TDF and ABC. eGFR was calculated using the equation from the 4-variable Modification of Diet in Renal Disease (MDRD) study, $eGFR = 186 \times [\text{serum creatinine}]^{-1.154} \times [\text{age}]^{-0.203} \times [0.742 \text{ if patient is female}] \times [1.212 \text{ if patient is African American}]$ [22]. In this study, the primary exposure variable was TDF use over ABC as part of the initial ART.

The potential risk factors for renal dysfunction were determined according to previous studies and collected together with the basic demographics from the medical records [15,23–25]. They included age, sex, body weight, body mass index, (BMI) = {body weight (kg) / [(height (m)]^2}, baseline laboratory data (CD4 cell count, HIV viral load, and serum creatinine), and presence or absence of other medical conditions (concurrent use of ritonavir-boosted protease inhibitors, concurrent nephrotoxic drugs such as ganciclovir, sulfamethoxazole/trimethoprim, and non-steroidal anti-inflammatory agents, diabetes mellitus defined by using anti-diabetic agents or fasting plasma glucose >126 mg/dl or plasma glucose >200 mg/dl on two different days, co-infection with hepatitis B defined by positive hepatitis B surface antigen, co-infection with hepatitis C defined by positive HCV viral load, hypertension defined by current treatment with antihypertensive agents or two successive measurements of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg at the clinic, dyslipidemia defined by current treatment with lipid-lowering agents, and current smoking). At our clinic, weight and blood pressure were measured on every visit whereas other variables were measured in the first visit and at least once annually. We used the data on or closest to and preceding the day of starting ART by no more than 90 days.

Statistical analysis

The time to 25% decline in eGFR from the baseline was calculated from the date of commencement of treatment to the date of diagnosis of the above-defined renal dysfunction. Censored cases represented those who discontinued ABC or TDF, dropped out, were referred to other facilities, or at the end of follow-up period. The time from the start of ART to >25% decrease in eGFR was analyzed by the Kaplan Meier method for patients who started TDF (TDF arm) and ABC (ABC arm), and the log-rank test was used to determine the statistical significance. The Cox proportional hazards regression analysis was used to estimate the

impact of TDF use over ABC on the incidence of more than 25% decrease in eGFR relative to the baseline. The impact of each basic demographics, baseline laboratory data, and other medical conditions listed above was also estimated with univariate Cox proportional hazards regression.

To estimate the unbiased prognostic impact of TDF use over ABC for renal dysfunction, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for TDF use over ABC. Model 2 included age and weight plus model 1 in order to adjust for basic characteristics. In model 3, we added variables with P values <0.05 in univariate analysis for adjustment (these included age per 1 year, weight per 1 kg decrement, CD4 count per 1 /μl decrement, HIV viral load per log10/ml, serum creatinine per 1 mg/dl, concurrent use of nephrotoxic drug(s), hepatitis B infection, and diabetes mellitus). The eGFR and the BMI were excluded from multivariate analysis because of their multicollinearity with age and serum creatinine, and weight, respectively, since eGFR and BMI are gained by the equation of those variables [22,26]. We chose to add weight instead of BMI because our previous work showed that weight was more useful and handy information to estimate the risk for TDF-related nephrotoxicity than BMI [16].

As a sensitivity analysis, creatinine clearance was similarly calculated with Cockcroft-Gault equation for each patient, creatinine clearance = [(140 - age) × weight (kg)] / (serum creatinine×72)(×0.85 for females) [27]. Actual body weight was used for the calculation. The impact of TDF use over ABC for >25% decrement of creatinine clearance from the baseline was estimated in univariate analysis and multivariate analysis adjusted with the before mentioned variables with Cox proportional hazards model.

To estimate the impact of weight on TDF-related nephrotoxicity, we did subgroup analysis for intertertile baseline body weight categories: ≤60, 61–68, and >68 kg. Then, the abovementioned multivariate analysis with eGFR was conducted for each subgroup.

We also used a repeated measures mixed model to estimate and compare changes in eGFR between ABC and TDF from baseline to 2 years after initiation of ART by 6-month intervals adjusted for baseline eGFR and weight [10]. For each patient, the eGFR values at closest to and preceding 24, 48, 72 and 96 weeks after commencement of ART were collected. In this analysis, censoring occurred at discontinuation of TDF or ABC, leaving care, or reaching the end of the observation period before 96 weeks. Sensitivity analysis with creatinine clearance calculated by Cockcroft-Gault equation was similarly conducted.

Statistical significance was defined at two-sided p values <0.05. We used hazard ratios (HRs) and 95% confidence intervals (95% CIs) to estimate the impact of each variable on renal dysfunction. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

The study subjects were 199 patients in the TDF arm and 304 patients in the ABC arm who fulfilled the abovementioned criteria. Table 1 shows the demographics, laboratory data, and medical conditions of the study population at baseline. The majority of the study population was males, comparatively young and had a small stature (median weight, 64 kg, median BMI, 22.2 kg/m^2). More than 80% of the patients in the two arms had ritonavir-boosted PI. In the ABC arm, patients had significantly lower CD4 count (p = 0.006), were significantly more likely to have hypertension (p<0.001), and tended to use more nephrotoxic drugs (p = 0.109). On the other hand, in the TDF arm, patients had marginally higher baseline eGFR (p = 0.098) and were significantly more likely to have hepatitis B virus infection (P<0.001). However, all other major background parameters were similar in the two groups (Table 1).

More than 25% decrement in eGFR from baseline occurred in 44 patients (22.1%) in the TDF arm and 41 (13.5%) in the ABC arm, with an estimated incidence of 9.84 and 4.55 per 100 person-years, respectively. Figure 1 shows the time from ART initiation to >25% decrease in eGFR by the Kaplan Meier method in the two groups. Patients who started TDF-containing ART were significantly more likely to develop renal dysfunction, compared to the ABC group (p = 0.001, Log-rank test). The median time from commencement of ART to occurrence of >25% decrement in eGFR was 246 days (range, 1–1,339 days) for the TDF arm and 501 days (range, 7–2,022) for ABC arm. The total observation period was 447.2 patient-years [median, 839 days, interquartile range (IQR), 357–1137 days] for the TDF arm and 901.7 patient-years (median, 1,119 days, IQR, 660.5–1509 days) for the ABC arm.

Univariate analysis showed a significant relationship between TDF use and >25% decrement in eGFR (HR = 1.747; 95%CI, 1.152–2.648; p = 0.009) (Table 2). Furthermore, old age, small body weight, low baseline CD4 count, high HIV viral load, high eGFR, low serum creatinine, concurrent use of nephrotoxic drugs, hepatitis B infection, and diabetes mellitus were associated with renal dysfunction. On the other hand, concurrent use of ritonavir boosted PIs was not associated with renal dysfunction (HR = 1.220; 95%CI, 0.663–2.244; p = 0.523). Multivariate analysis identified TDF use as a significant risk for >25% decrement in eGFR after adjustment for age and weight (adjusted HR = 1.893; 95%CI, 1.243–2.881; p<0.003) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR = 2.080; 95%CI, 1.339–3.232; p<0.001) (Table 3, Model 3). We also conducted a sensitivity analysis using BMI decrement instead of weight as a variable in Table 3, Model 3. The results were almost identical; TDF use over ABC use was a risk for renal dysfunction (adjusted HR 1.957, 95% CI 1.262–3.036, p = 0.003).

Sensitivity analysis with creatinine clearance confirmed the abovementioned findings: both univariate and multivariate analyses showed that TDF use was significantly associated with >25% decrement in eGFR (univariate analysis: HR = 2.212; 95%CI, 1.340–3.653; p = 0.002) (multivariate analysis: adjusted HR = 2.544; 95%CI, 1.493–4.335; p = 0.001).

Subgroup analysis of the patients stratified by intertertile baseline body weight showed that the lower the baseline body weight, the more evident the impact of TDF on renal dysfunction (≤60 kg: adjusted HR = 2.771; 95%CI, 1.494–5.139; p = 0.001) (61–68 kg: adjusted HR = 1.908; 95%CI, 0.764–4.768; p = 0.167) (>68 kg: adjusted HR = 0.997; 95%CI, 0.318–3.121; p = 0.995) (Table 4). These findings suggest that there is the effect modification by baseline body weight on TDF-associated renal dysfunction.

Data analysis by repeated measures mixed models showed a significant decrease in adjusted mean eGFR from the baseline to 96 weeks in both groups (TDF: -9.984 ml/min/1.73m^2, 95%CI -12.05 to -7.914 ml/min/1.73m^2, p<0.001; ABC: -5.393 ml/min/1.73m^2, 95%CI -7.087 to -3.699 ml/min/1.73m^2, p<0.001) (Figure 2). There was a statistically significant interaction between the two arms over time (p = 0.003), indicating that adjusted mean eGFR decreased more significantly in the TDF group than in the ABC group after initiation of ART. Analysis of eGFR in each group demonstrated a rapid decrease during the first 24 weeks,

Table 1. Baseline demographics and laboratory data of patients who received tenofovir- and abacavir-based antiretroviral therapy (n = 503).

	TDF (n = 199)	ABC (n = 304)	P value
Sex (male), n (%)	196 (98.5)	296 (97.4)	0.539
Median (IQR) age	36 (31–44)	37 (31–43)	0.436
Median (IQR) weight (kg)	64 (58–69)	64 (58.0–70.9)	0.426
Median (IQR) BMI (kg/m^2)	22.1 (20.4–23.9)	22.2 (20.3–24.6)	0.321
Median (IQR) eGFR (ml/min/1.73m^2)	119.4 (103.0–135.0)	115.6 (102.4–132.2)	0.098
Median (IQR) serum creatinine (mg/dl)	0.74 (0.67–0.84)	0.75 (0.68–0.83)	0.250
Median (IQR) CD4 count (/μl)	199 (109–272)	178.5 (75.3–234.8)	0.006
Median (IQR) HIV RNA viral load (log10/ml)	4.63 (4.20–5.20)	4.74 (4.23–5.20)	0.731
Ritonavir-boosted protease inhibitors, n (%)	173 (86.9)	256 (84.2)	0.441
Protease inhibitors (unboosted), n (%)	5 (2.5)	20 (6.6)	0.038
NNRTIs, n (%)	16 (8.0)	26 (8.6)	0.848
INIs, n (%)	5 (2.5)	2 (0.7)	0.119
Hypertension, n (%)	5 (2.5)	53 (17.4)	<0.001
Dyslipidemia, n (%)	4 (2.0)	4 (1.3)	0.718
Diabetes mellitus, n (%)	8 (4.0)	12 (3.9)	1.000
Concurrent use of nephrotoxic drugs, n (%)	65 (32.7)	121 (39.8)	0.109
Hepatitis B, n (%)	35 (17.6)	9 (3.0)	<0.001
Hepatitis C, n (%)	7 (3.5)	7 (2.3)	0.421
Current smoker, n (%)	93 (46.7)	149 (49.3)	0.585

TDF: tenofovir, ABC: abacavir, IQR: interquartile range, BMI: body mass index, eGFR: estimated glomerular filtration rate, NNRTI: non- nucleoside reverse transcriptase inhibitor, INI: integrase inhibitor.
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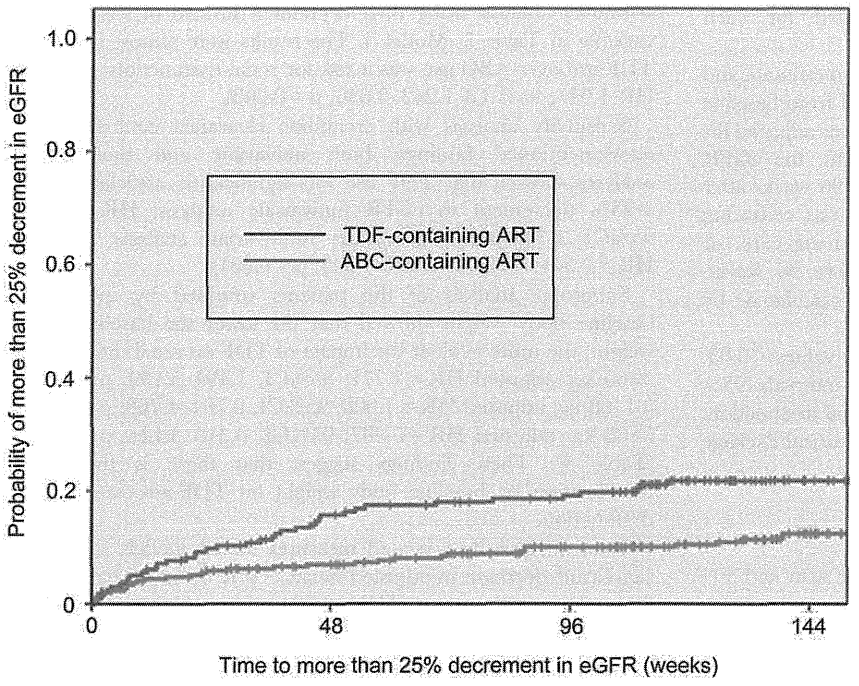

Figure 1. Kaplan-Meier curve showing the time to renal dysfunction in patients treated with TDF or ABC. Compared to treatment-naïve patients who commenced treatment with ABC, those on TDF were more likely to develop >25% fall in eGFR (p = 0.001, Log-rank test). TDF: tenofovir, ABC: abacavir, ART: antiretroviral therapy, eGFR: estimated glomerular filtration rate.
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Table 2. Univariate analysis to estimate the risk of various factors in inducing more than 25% fall in eGFR.

	Hazard ratio	95% CI	P value
TDF vs. ABC use	1.747	1.152–2.648	0.009
Female gender	0.048	0.000–16.93	0.310
Age per 1 year	1.031	1.011–1.051	0.002
Weight per 1 kg decrement	1.047	1.023–1.072	<0.001
BMI per 1 kg/m^2 decrement	1.152	1.066–1.244	<0.001
CD4 count per 1 /μl decrement	1.006	1.004–1.008	<0.001
HIV viral load per log10/ml	1.562	1.179–2.071	0.002
Ritonavir-boosted protease inhibitors	1.220	0.663–2.244	0.523
Baseline eGFR per 1 ml/min/1.73m^2	1.009	1.005–1.014	<0.001
Baseline serum creatinine per 1mg/dl	0.016	0.003–0.086	<0.001
Concurrent nephrotoxic drug	2.134	1.417–3.214	<0.001
Hepatitis B	1.866	1.038–3.356	0.037
Hepatitis C	1.721	0.631–4.695	0.289
Diabetes mellitus	2.558	1.181–5.540	0.017
Hypertension	0.865	0.448–1.669	0.664
Current smoking	0.989	0.657–1.489	0.958

eGFR: estimated glomerular filtration rate, CI: confidence interval, TDF: tenofovir, ABC: abacavir, BMI: body mass index.
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followed by a plateau until 96 weeks. In sensitivity analysis with creatinine clearance calculated by Cockcroft-Gault equation, the result was the same; a significant decrease from the baseline to 96 weeks in both groups (TDF: -10.62 ml/min, 95%CI -13.78 to -7.458 ml/min; ABC: -4.325 ml/min, 95%CI -6.893 to -1.756 ml/min) and significantly more eGFR decrement in the TDF group ($p = 0.019$).

Discussion

In this observational Japanese cohort, treatment-naïve patients who started TDF-containing ART experienced eGFR decline of >25% approximately twice as likely compared to those treated with ABC-containing regimen. Univariate and multivariate analyses identified TDF use as an independent risk factor for renal dysfunction. Subgroup analysis showed that the effect of TDF on renal dysfunction was more evident in patients with lower body weight. Furthermore, eGFR decrement was significantly larger in the TDF group than in ABC group over the 2-year observation period.

In our previous study, we demonstrated a high incidence of TDF-associated nephrotoxicity in patients with low body weight, and the use of a robust statistical model indicated a greater decline in renal function in patients of low body weight treated with TDF [16]. The results of the present study further emphasize the importance of low body weight as a risk factor for TDF-related nephrotoxicity by showing that in a cohort of patients with low body weight, the incidence of renal dysfunction was twice higher with TDF use than with ABC use.

Among the studies designed to compare renal function after the commencement of TDF and ABC-containing ART for treatment-naïve patients, our cohort had the lowest median body weight (64 kg). This is lower than the median body weight of patients of the ASSERT study conducted in European countries (72 kg) [10]. The

Table 4. Multivariate analysis to estimate the risk of TDF- over ABC-based antiretroviral therapy in the induction of more than 25% fall in eGFR according to baseline body weight.

	Adjusted HR	95% CI	P value
Baseline body weight ≤60 kg (n = 171)			
TDF vs. ABC use	2.771	1.494–5.139	0.001
Baseline body weight 61–68 kg (n = 167)			
TDF vs. ABC use	1.908	0.764–4.768	0.168
Baseline body weight >68 kg (n = 165)			
TDF vs. ABC use	0.997	0.318–3.121	0.995

TDF use was adjusted with the same variables indicated in Model 3, Table 3: age per 1 year, weight per 1 kg decrement, CD4 count per 1 /μl decrement, HIV viral load per log10/ml, serum creatinine per 1 mg/dl, concurrent use of nephrotoxic drugs, hepatitis B infection, and diabetes mellitus.
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Table 3. Multivariate analysis to estimate the risk of TDF- over ABC-based antiretroviral therapy in inducing more than 25% fall in eGFR.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95% CI	HR	95%CI	HR	95%CI
TDF vs. ABC use†	1.747	1.152–2.648	1.893	1.243–2.881	2.080	1.339–3.232
Age per 1 year			1.029	1.010–1.048	1.020	1.000–1.040
Weight per 1 kg decrement†			1.046	1.022–1.071	1.028	1.005–1.052
CD4 count per 1 /μl decrement†					1.004	1.002–1.007
HIV viral load per log10/ml					1.048	0.749–1.466
Serum creatinine per 1 mg/dl†					0.053	0.009–0.304
Use of nephrotoxic drug					1.309	0.825–2.077
Hepatitis B					1.070	0.573–2.000
Diabetes mellitus					1.565	0.684–3.582

†P<0.05 in Model 3.
TDF: tenofovir, ABC: abacavir, eGFR: estimated glomerular filtration rate, HR: hazard ratio, CI: confidence interval.
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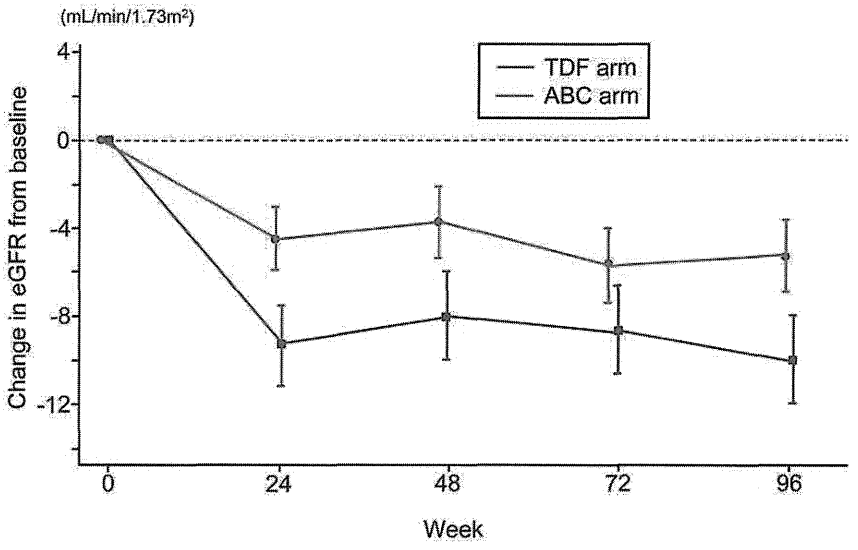

Figure 2. Changes in eGFR in patients treated with TDF or ABC between baseline and 96 weeks. The fall in eGFR was significantly greater in the TDF group than the ABC group (p = 0.003). Data are adjusted mean ±95% confidence interval. eGFR: estimated glomerular filtration rate, TDF: tenofovir, ABC: abacavir.
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results of the present study on TDF-related nephrotoxicity differ from the findings of randomized clinical trials that demonstrated no major change in renal function of TDF- and ABC-treated patients over 48–96 week follow-up [2,10,11]. The discrepant results might arise from differences between observational cohort and clinical trials, since observational studies tend to express the results in "real world setting" whereas clinical trials include patients who fulfill more strict criteria, therefore with better profile [9]. The discrepant results could be also due to the use of different definitions for renal dysfunction in these studies. However, the discrepant results could also reflect the difference in median body weight between the present study and these clinical trials. The results of our subgroup analysis support this hypothesis by showing that the effect of TDF on renal dysfunction was more evident in patients with low body weight. Apart from being low-body-weighted, the patients in this study did not appear to have many of other established risks for TDF-related nephrotoxicity; they were comparatively young, had relatively stable CD4 count, and had only a few co-morbidities (Table 1). Although the majority concurrently used ritonavir-boosted PIs, which are a probable risk for TDF-related nephrotoxicity, ritonavir-boosted PIs were not significantly associated with renal dysfunction in our cohort (Table 2) [24].

Changes in eGFR in those patients treated with TDF-containing ART were characterized by a rapid decline during the first 24 weeks of therapy, followed by a plateau until 96 weeks (Fig. 2). This finding is consistent with that reported from the Johns Hopkins group [9,28]. Together with the finding that the median time from commencement of ART to the >25% decline in eGFR in the TDF-treated patients was 246 days, these results suggest that careful monitoring of renal function is particularly warranted in the first year of TDF-based therapy. Thus, we suggest that renal function should be monitored by measurement of serum creatinine at least once annually in resource-limited settings and twice annually in resource-rich settings in patients starting TDF-containing ART, especially those with baseline body weight <60 kg.

The Department of Health and Human Services guideline for the treatment of HIV infection in the U.S. lists ABC as an alternative NRTI because it can potentially cause serious hypersensitivity reaction and cardiovascular diseases (URL:http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). However, some international guidelines consider both TDF and ABC as the preferred NRTIs under the condition that ABC should be used with caution in patients with viral load >100,000 copies/mL, based on the low incidence of ABC-related hypersensitivity among HLA-B*5701-negative population and the controversial association between ABC and cardiovascular diseases [1,29–32] (URL: http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf) (http://www.haart-support.jp/guideline2011.pdf. in Japanese). The present study, together with our previous analysis that demonstrated preferential TDF-related nephrotoxicity in patients with low body weight, emphasize the advantage of ABC over TDF with regard to prognosis of renal function in low body weight patients [16].

TDF is the prodrug of acyclic nucleotide analog tenofovir, which is excreted by both glomerular filtration and active tubular secretion. Tenofovir is considered to cause mitochondrial damage in proximal renal tubular cells [33]. The concentration of tenofovir in the proximal renal tubules could be augmented with the complex interactions of pharmacological, environmental, and genetic factors, including small body weight, consequently resulting in renal tubular dysfunction [34]. Body weight has been identified as an important factor in TDF-related nephrotoxicity not only in clinical trials, but also in *in vitro* and pharmacokinetic studies [35–37].

The present study has several limitations. First, because of its retrospective nature, it was not possible to control the baseline characteristics of the enrolled patients. Thus, it is possible that patients with potential risk for TDF-related nephrotoxicity were not prescribed TDF. A proportion of patients treated with ABC had low CD4 count and others were hypertensive, both conditions are known risk factors for renal dysfunction [23,25]. However, for these reasons, the incidence of TDF-associated renal dysfunction might have been underestimated. Second, the definition of TDF-related nephrotoxicity, especially the criteria used to evaluate proximal renal tubular damage, is not uniformly established in the field and is different in the published studies. Accordingly, we