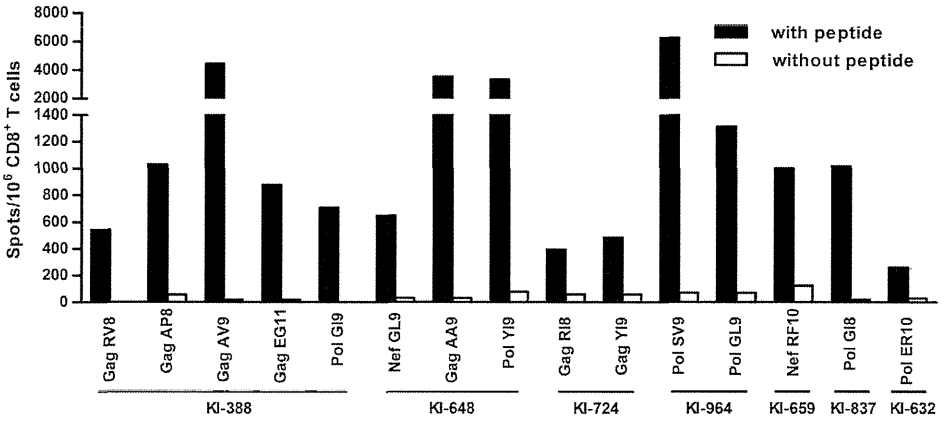

Fig. 5. $CD8^+$ T cell responses to clade B-drived epitope peptides in HIV-1 clade A/E-infected Japanese individuals $CD8^+$ T cell responses to 15 clade B-derived epitope peptides were analyzed by performing ELISPOT assay using $CD8^+$ T cells from seven clade A/E-infected individuals (KI-388, KI-632, KI-648, KI-659, KI-724, KI-837, and KI-964). >200 spots were evaluated as positive response.

and presented in cells infected with these viruses. On the other hand, $CD8^+$ bulk T cells induced by Pol FR9 recognized the clade B virus-infected cells but failed to recognize the clade A/E virus-infected cells (Fig. 4B). This finding is consistent with the low ability of these cells to recognize the clade A/E peptide (Fig. 4A). In contrast, $CD8^+$ T cells induced by Nef GL9 recognized the clade A/E virus-infected cells but failed to recognize the clade B virus-infected cells although these T cells could recognize GL9 peptide. This result may be explained by the fact that the amino acid sequence of the clade B consensus peptide is different than that of the clade B clone, NL4-3(Ala and Val at position 1 and 3, respectively, in Nef GL9 region). Thus, $CD8^+$ T cells induced by 8 out of 10 clade B-derived epitope peptides successfully recognized both the clade B virus-infected and clade A/E-infected cells.

3.4. Detection of cross-clade $CD8^+$ T cell responses in the clade A/E-infected Japanese individuals

To confirm $CD8^+$ T cell responses to the 15 epitopes including Nef GL9, we analyzed $CD8^+$ T cell responses to the clade B-derived epitope peptides in clade A/E-infected individuals who had HLA alleles restricting these epitopes. Positive $CD8^+$ T cell responses to these 15 clade B-derived epitope peptides were detected in PBMCs from chronically HIV-1 clade A/E-infected individuals (Fig. 5). These results indicate that these cross-clade CTLs are elicited in these individuals.

4. Discussion

Previous studies, which focused on known CTL epitopes for the clade B or C viruses, showed the existence of cross-clade CTLs in HIV-1-infected individuals by demonstrating that CTL clones established by using clade-matched peptides from the clade B-infected or the clade C-infected individuals recognize the cells infected with other clade viruses [13–17]. These studies also showed that conserved epitopes across the clades are more likely recognized by the T cell clones and suggested that conserved epitopes would be a more preferable target for a widely effective CTL vaccine than variable ones. In the present study, we for the first time performed a comprehensive analysis of cross-clade $CD8^+$ T cells by using 11-mer overlapping clade B-derived peptides to stimulate $CD8^+$ T cells from HIV-1 clade A/E-infected individuals. Interestingly, we found a similar level of $CD8^+$ T cell responses to clade B-derived Nef, Gag, and Pol peptides in the clade A/E virus-infected individuals as compared to those to the same peptides in clade B-infected individuals. These results strongly suggested the existence of a high number of cross-clade CTLs in the clade A/E virus-infected individuals. Indeed, we finally identified 15 cross-clade CTL epitopes from only 13 out of 85 overlapping peptide cocktails. These results strongly suggest that a large number of cross-clade CTLs were elicited in the clade A/E virus-infected individuals.

$CD8^+$ T cells induced by Pol FR9 recognized to a much lesser extent the clade A/E-derived peptide (FR9-8S) than the clade B-derived peptide and recognized cells infected with

T cells were induced from KI-837. **A.** Cross-recognition of HIV-1 clade B (open circle) and clade A/E (closed square) optimal epitope peptides of the consensus sequence. These analyses were performed at peptide concentrations from 0.1 to 100 nM. **B.** Cross-recognition of cells infected with clade B or clade A/E virus. IFN-γ production by $CD8^+$ bulk T cells in response to HLA-positive cells prepulsed with clade B or clade A/E consensus optimal peptide and that in response to HLA-negative cells infected with the virus and to uninfected HLA-positive cells were measured as positive and negative controls, respectively. All epitope sequences derived from the clade B or the clade A/E were identical to the sequences from clone virus (NL4-3 or 93JP-NH1) except for clade B Nef GL9 epitope (GALDLSHFL). NL4-3 has Ala and Val at positions 1 and 3 of this epitope, respectively.

clade B virus but not those infected with A/E viruses, suggesting that PolFR9-8S was not an epitope. Indeed, the HLA-A*33:03$^+$ individuals were infected with the clade A/E virus carrying Pol FR9 sequence but not Pol FR9-8S one (data not shown). The CD8$^+$ T cells induced by Nef GL9 recognized the clade A/E virus-infected cells, whereas they failed to recognize the clade B-infected ones. These T cells could recognize GL9 peptide, though they recognized more effectively Nef GL9-3F-7F peptides than the Nef GL9 one. However, CD8$^+$ T cells specific for both Nef GL9 and Nef GL9-3F-7F were detected in 3 of 7 HLA-A*02:06$^+$ individuals (data not shown). These results suggest that Nef GL9-3F-7F had been presented in the clade A/E-infected individuals. Therefore, the failure of the T cells to recognize cells infected with NL4-3 virus may have resulted from a different amino acid sequence of this epitope between the clade B consensus peptide and NL4-3 (Ala and Val at position 1 and 3, respectively, in Nef GL9 region). CD8$^+$ bulk T cells induced by 8 other diverse epitopes effectively recognized both the clade B-infected and the clade A/E-infected cells, suggesting that these diverse epitopes could be cross-recognized by the T cells.

We previously reported that Phe at position 2 of Nef RF10 is an escape mutation in the clade B virus [48]. This escape mutation was frequently found in the clade A/E virus, though the consensus sequence was RF10-5C (RYPLCFGWCF; Table 1). Since RF10 and RF10-5C were cross-recognized by the CD8$^+$ T cells induced by the RF10 peptide, these T cells would be expected to select 2F mutants in the clade A/E-infected individuals. These results indicate that RF10-5C was an HLA-A*24:02-restricted epitope in the clade A/E-infected individuals and that RF10-5C-specific CD8$^+$ T cells could cross-recognize the RF10 epitope.

Since these epitopes were restricted by Asian HLA alleles, vaccine targeting these epitopes can cover Asian countries including south-east Asia and China where clade A/E and clade B viruses are prevalent. An HLA-B*40:02-restricted Nef epitope was known to be presented by world-wise HLA allele HLA-B*40:01 [33]. In addition, a previous study showed that Pol GL9-specific CD8$^+$ T cells were elicited in a vaccinated individual carrying world-wise HLA allele, HLA-A*02:01 [42]. These studies together suggest that some of the HLA-B*40:02-resticted and HLA-A*02:06-restricted epitopes identified in this study may be CTL epitopes presented by these world-wise HLA alleles. Thus, vaccine targeting the cross-clade epitopes identified in this study may cover countries in Europe, and northern and southern Americas in addition to Asian countries.

In conclusion, we here performed the first comprehensive study of cross-clade T cell responses and demonstrated that CD8$^+$ T cell responses to clade B-derived Nef, Gag, and Pol peptides were successfully induced in the clade A/E virus-infected individuals. We finally identified the 15 cross-clade epitopes which include not only conserved epitopes but also polymorphic epitopes across the different clades. These epitopes can thus be candidate targets of CTL-based vaccines.

Acknowledgments

The authors thank Dr. T. Akahoshi and Rie Maruyama for technical assistance and Sachiko Sakai for her secretarial assistance.

This research was supported by the Global COE program "Global Education and Research Center Aiming at the control of AIDS," launched as a project commissioned by the Ministry of Education, Science, Sports, and Culture, Japan; and by a Joint Research Grant with the Institute of Tropical Medicine, Nagasaki University.

References

[1] B.D. Walker, B.T. Korber, Immune control of HIV: the obstacles of HLA and viral diversity, Nat. Immunol. 2 (2001) 473–475.

[2] S. Rerks-Ngarm, P. Pitisuttithum, S. Nitayaphan, J. Kaewkungwal, J. Chiu, R. Paris, N. Premsri, C. Namwat, M. de Souza, E. Adams, M. Benenson, S. Gurunathan, J. Tartaglia, J.G. McNeil, D.P. Francis, D. Stablein, D.L. Birx, S. Chunsuttiwat, C. Khamboonruang, P. Thongcharoen, M.L. Robb, N.L. Michael, P. Kunasol, J.H. Kim, MOPHTAVEG investigators, Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand, N. Engl. J. Med. 361 (2009) 2209–2220.

[3] J. Hemelaar, E. Gouws, P.D. Ghys, S. Osmanov, WHO-UNAIDS Network for HIV isolation and characterisation, global trends in molecular epidemiology of HIV-1 during 2000-2007, AIDS 25 (2011) 679–689.

[4] M.S. de Souza, S. Ratto-Kim, W. Chuenarom, A. Schuetz, S. Chantakulkij, B. Nuntapinit, A. Valencia-Micolta, D. Thelian, S. Nitayaphan, P. Pitisuttithum, R.M. Paris, J. Kaewkungwal, N.L. Michael, S. Rerks-Ngarm, B. Mathieson, M. Marovich, J.R. Currier, J.H. Kim, Ministry of Public Health-Thai AIDS Vaccine Evaluation Group Collaborators, The Thai phase III trial (RV144) vaccine regimen induces T cell responses that preferentially target epitopes within the V2 region of HIV-1 envelope, J. Immunol. 188 (2012) 5166–5176.

[5] M. Bonsignori, J. Pollara, M.A. Moody, M.D. Alpert, X. Chen, K.K. Hwang, P.B. Gilbert, Y. Huang, T.C. Gurley, D.M. Kozink, D.J. Marshall, J.F. Whitesides, C.Y. Tsao, J. Kaewkungwal, S. Nitayaphan, P. Pitisuttithum, S. Rerks-Ngarm, J.H. Kim, N.L. Michael, G.D. Tomaras, D.C. Montefiori, G.K. Lewis, A. DeVico, D.T. Evans, G. Ferrari, H.X. Liao, B.F. Haynes, Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family, J. Virol. 86 (2012) 11521–11532.

[6] B.F. Haynes, P.B. Gilbert, M.J. McElrath, S. Zolla-Pazner, G.D. Tomaras, S.M. Alam, D.T. Evans, D.C. Montefiori, C. Karnasuta, R. Sutthent, H.X. Liao, A.L. DeVico, G.K. Lewis, C. Williams, A. Pinter, Y. Fong, H. Janes, A. DeCamp, Y. Huang, M. Rao, E. Billings, N. Karasavvas, M.L. Robb, V. Ngauy, M.S. de Souza, R. Paris, G. Ferrari, R.T. Bailer, K.A. Soderberg, C. Andrews, P.W. Berman, N. Frahm, S.C. De Rosa, M.D. Alpert, N.L. Yates, X. Shen, R.A. Koup, P. Pitisuttithum, J. Kaewkungwal, S. Nitayaphan, S. Rerks-Ngarm, N.L. Michael, J.H. Kim, Immune-correlates analysis of an HIV-1 vaccine efficacy trial, N. Engl. J. Med. 366 (2012) 1275–1286.

[7] P. Kiepiela, K. Ngumbela, C. Thobakgale, D. Ramduth, I. Honeyborne, E. Moodley, S. Reddy, C. de Pierres, Z. Mncube, N. Mkhwanazi, K. Bishop, M. van der Stok, K. Nair, N. Khan, H. Crawford, R. Payne, A. Leslie, J. Prado, A. Prendergast, J. Frater, N. McCarthy, C. Brander, G.H. Learn, D. Nickle, C. Rousseau, H. Coovadia, J.I. Mullins, D. Heckerman, B.D. Walker, P. Goulder, CD8+ T-cell responses to different HIV proteins have discordant associations with viral load, Nat. Med. 13 (2007) 46–53.

[8] R. Zuniga, A. Lucchetti, P. Galvan, S. Sanchez, C. Sanchez, A. Hernandez, H. Sanchez, N. Frahm, C.H. Linde, H.S. Hewitt,

W. Hildebrand, M. Altfeld, T.M. Allen, B.D. Walker, B.T. Korber, T. Leitner, J. Sanchez, C. Brander, Relative dominance of Gag p24-specific cytotoxic T lymphocytes is associated with human immunodeficiency virus control, J. Virol. 80 (2006) 3122–3125.

[9] S.A. Migueles, M.S. Sabbaghian, W.L. Shupert, M.P. Bettinotti, F.M. Marincola, L. Martino, C.W. Hallahan, S.M. Selig, D. Schwartz, J. Sullivan, M. Connors, HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors, Proc. Natl. Acad. Sci. USA 97 (2000) 2709–2714.

[10] E.L. Turnbull, A.R. Lopes, N.A. Jones, D. Cornforth, P. Newton, D. Aldam, P. Pellegrino, J. Turner, I. Williams, C.M. Wilson, P.A. Goepfert, M.K. Maini, P. Borrow, HIV-1 epitope-specific CD8+ T cell responses strongly associated with delayed disease progression cross-recognize epitope variants efficiently, J. Immunol. 176 (2006) 6130–6146.

[11] S.L. Rowland-Jones, T. Dong, K.R. Fowke, J. Kimani, P. Krausa, H. Newell, T. Blanchard, K. Ariyoshi, J. Oyugi, E. Ngugi, J. Bwayo, K.S. MacDonald, A.J. McMichael, F.A. Plummer, Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi, J. Clin. Invest. 102 (1998) 1758–1765.

[12] R.P. Johnson, A. Trocha, L. Yang, G.P. Mazzara, D.L. Panicali, T.M. Buchanan, B.D. Walker, HIV-1 gag-specific cytotoxic T lymphocytes recognize multiple highly conserved epitopes. Fine specificity of the gag-specific response defined by using unstimulated peripheral blood mononuclear cells and cloned effector cells, J. Immunol. 147 (1991) 1512–1521.

[13] D.M. McKinney, R. Skvoretz, B.D. Livingston, C.C. Wilson, M. Anders, R.W. Chesnut, A. Sette, M. Essex, V. Novitsky, M.J. Newman, Recognition of variant HIV-1 epitopes from diverse viral subtypes by vaccine-induced CTL, J. Immunol. 173 (2004) 1941–1950.

[14] M.S. Bennett, H.L. Ng, A. Ali, O.O. Yang, Cross-clade detection of HIV-1-specific cytotoxic T lymphocytes does not reflect cross-clade antiviral activity, J. Infect Dis. 197 (2008) 390–397.

[15] K. Fukada, H. Tomiyama, C. Wasi, T. Matsuda, S. Kusagawa, H. Sato, S. Oka, Y. Takebe, M. Takiguchi, Cytotoxic T-cell recognition of HIV-1 cross-clade and clade-specific epitopes in HIV-1-infected Thai and Japanese patients, AIDS 16 (2002) 701–711.

[16] H. Cao, P. Kanki, J.L. Sankale, A. Dieng-Sarr, G.P. Mazzara, S.A. Kalams, B. Korber, S. Mboup, B.D. Walker, Cytotoxic T-lymphocyte cross-reactivity among different human immunodeficiency virus type 1 clades: implications for vaccine development, J. Virol. 71 (1997) 8615–8623.

[17] D. Durali, J. Morvan, F. Letourneur, D. Schmitt, N. Guegan, M. Dalod, S. Saragosti, D. Sicard, J.P. Levy, E. Gomard, Cross-reactions between the cytotoxic T-lymphocyte responses of human immunodeficiency virus-infected African and European patients, J. Virol. 72 (1998) 3547–3553.

[18] H. Chen, Z.M. Ndhlovu, D. Liu, L.C. Porter, J.W. Fang, S. Darko, M.A. Brockman, T. Miura, Z.L. Brumme, A. Schneidewind, A. Piechocka-Trocha, K.T. Cesa, J. Sela, T.D. Cung, I. Toth, F. Pereyra, X.G. Yu, D.C. Douek, D.E. Kaufmann, T.M. Allen, B.D. Walker, TCR clonotypes modulate the protective effect of HLA class I molecules in HIV-1 infection, Nat. Immunol. 13 (2012) 691–700.

[19] M.P. Sanou, A.S. De Groot, M. Murphey-Corb, J.A. Levy, J.K. Yamamoto, HIV-1 vaccine trials: evolving concepts and designs, Open AIDS J. 6 (2012) 274–288.

[20] B.T. Korber, N.L. Letvin, B.F. Haynes, T-cell vaccine strategies for human immunodeficiency virus, the virus with a thousand faces, J. Virol. 83 (2009) 8300–8314.

[21] A.M. Masemola, T.N. Mashishi, G. Khoury, H. Bredell, M. Paximadis, T. Mathebula, D. Barkhan, A. Puren, E. Vardas, M. Colvin, L. Zijenah, D. Katzenstein, R. Musonda, S. Allen, N. Kumwenda, T. Taha, G. Gray, J. McIntyre, S.A. Karim, H.W. Sheppard, C.M. Gray, HIVNET 028 Study Team, Novel and promiscuous CTL epitopes in conserved regions of Gag targeted by individuals with early subtype C HIV type 1 infection from southern Africa, J. Immunol. 173 (2004) 4607–4617.

[22] Y. Roshorm, M.G. Cottingham, M.J. Potash, D.J. Volsky, T. Hanke, T cells induced by recombinant chimpanzee adenovirus alone and in prime-boost regimens decrease chimeric EcoHIV/NDK challenge virus load, Eur. J. Immunol. 42 (2012) 3243–3255.

[23] M.L. Knudsen, A. Mbewe-Mvula, M. Rosario, D.X. Johansson, M. Kakoulidou, A. Bridgeman, A. Reyes-Sandoval, A. Nicosia, K. Ljungberg, T. Hanke, P. Liljestrom, Superior induction of T cell responses to conserved HIV-1 regions by electroporated alphavirus replicon DNA compared to that with conventional plasmid DNA vaccine, J. Virol. 86 (2012) 4082–4090.

[24] M. Rosario, N. Borthwick, G.B. Stewart-Jones, A. Mbewe-Mvula, A. Bridgeman, S. Colloca, D. Montefiori, A.J. McMichael, A. Nicosia, E.D. Quakkelaar, J.W. Drijfhout, C.J. Melief, T. Hanke, Prime-boost regimens with adjuvanted synthetic long peptides elicit T cells and antibodies to conserved regions of HIV-1 in macaques, AIDS 26 (2012) 275–284.

[25] Y. Roshorm, J.P. Hong, N. Kobayashi, A.J. McMichael, D.J. Volsky, M.J. Potash, M. Takiguchi, T. Hanke, Novel HIV-1 clade B candidate vaccines designed for HLA-B*5101(+) patients protected mice against chimaeric ecotropic HIV-1 challenge, Eur. J. Immunol. 39 (2009) 1831–1840.

[26] I. Cebere, L. Dorrell, H. McShane, A. Simmons, S. McCormack, C. Schmidt, C. Smith, M. Brooks, J.E. Roberts, S.C. Darwin, P.E. Fast, C. Conlon, S. Rowland-Jones, A.J. McMichael, T. Hanke, Phase I clinical trial safety of DNA- and modified virus Ankara-vectored human immunodeficiency virus type 1 (HIV-1) vaccines administered alone and in a prime-boost regime to healthy HIV-1-uninfected volunteers, Vaccine 24 (2006) 417–425.

[27] N. Goonetilleke, S. Moore, L. Dally, N. Winstone, I. Cebere, A. Mahmoud, S. Pinheiro, G. Gillespie, D. Brown, V. Loach, J. Roberts, A. Guimaraes-Walker, P. Hayes, K. Loughran, C. Smith, J. De Bont, C. Verlinde, D. Vooijs, C. Schmidt, M. Boaz, J. Gilmour, P. Fast, L. Dorrell, T. Hanke, A.J. McMichael, Induction of multifunctional human immunodeficiency virus type 1 (HIV-1)-specific T cells capable of proliferation in healthy subjects by using a prime-boost regimen of DNA- and modified vaccinia virus Ankara-vectored vaccines expressing HIV-1 Gag coupled to CD8+ T-cell epitopes, J. Virol. 80 (2006) 4717–4728.

[28] M. Mwau, I. Cebere, J. Sutton, P. Chikoti, N. Winstone, E.G. Wee, T. Beattie, Y.H. Chen, L. Dorrell, H. McShane, C. Schmidt, M. Brooks, S. Patel, J. Roberts, C. Conlon, S.L. Rowland-Jones, J.J. Bwayo, A.J. McMichael, T. Hanke, A human immunodeficiency virus 1 (HIV-1) clade A vaccine in clinical trials: stimulation of HIV-specific T-cell responses by DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines in humans, J. Gen. Virol. 85 (2004) 911–919.

[29] G.J. Gorse, L.R. Baden, M. Wecker, M.J. Newman, G. Ferrari, K.J. Weinhold, B.D. Livingston, T.L. Villafana, H. Li, E. Noonan, N.D. Russell, HIV Vaccine Trial Network, Safety and immunogenicity of cytotoxic T-lymphocyte poly-epitope, DNA plasmid (EP HIV-1090) vaccine in healthy, human immunodeficiency virus type 1 (HIV-1)-uninfected adults, Vaccine 26 (2008) 215–223.

[30] P. Spearman, S. Kalams, M. Elizaga, B. Metch, Y.L. Chiu, M. Allen, K.J. Weinhold, G. Ferrari, S.D. Parker, M.J. McElrath, S.E. Frey, J.D. Fuchs, M.C. Keefer, M.D. Lubeck, M. Egan, R. Braun, J.H. Eldridge, B.F. Haynes, L. Corey, NIAID HIV Vaccine Trials Network, Safety and immunogenicity of a CTL multiepitope peptide vaccine for HIV with or without GM-CSF in a phase I trial, Vaccine 27 (2009) 243–249.

[31] D. Salmon-Ceron, C. Durier, C. Desaint, L. Cuzin, M. Surenaud, N.B. Hamouda, J.D. Lelievre, B. Bonnet, G. Pialoux, I. Poizot-Martin, J.P. Aboulker, Y. Levy, O. Launay, ANRS VAC trial group, immunogenicity and safety of an HIV-1 lipopeptide vaccine in healthy adults: a phase 2 placebo-controlled ANRS trial, AIDS 24 (2010) 2211–2223.

[32] W. Jaoko, F.N. Nakwagala, O. Anzala, G.O. Manyonyi, J. Birungi, A. Nanvubya, F. Bashir, K. Bhatt, H. Ogutu, S. Wakasiaka, L. Matu, W. Waruingi, J. Odada, M. Oyaro, J. Indangasi, J. Ndinya-Achola, C. Konde, E. Mugisha, P. Fast, C. Schmidt, J. Gilmour, T. Tarragona, C. Smith, B. Barin, L. Dally, B. Johnson, A. Muluubya, L. Nielsen, P. Hayes, M. Boaz, P. Hughes, H. Hanke, A. McMichael, J. Bwayo, P. Kaleebu, Safety and immunogenicity of recombinant low-dosage HIV-1 A vaccine candidates vectored by plasmid pTHr DNA or modified

vaccinia virus Ankara (MVA) in humans in East Africa, Vaccine 26 (2008) 2788–2795.
[33] T. Watanabe, H. Murakoshi, H. Gatanaga, M. Koyanagi, S. Oka, M. Takiguchi, Effective recognition of HIV-1-infected cells by HIV-1 integrase-specific HLA-B *4002-restricted T cells, Microbe. Infect. 13 (2011) 160–166.
[34] S. Karaki, A. Kariyone, N. Kato, K. Kano, Y. Iwakura, M. Takiguchi, HLA-B51 transgenic mice as recipients for production of polymorphic HLA-A, B-specific antibodies, Immunogenetics 37 (1993) 139–142.
[35] H. Koizumi, T. Iwatani, J. Tanuma, M. Fujiwara, T. Izumi, S. Oka, M. Takiguchi, Escape mutation selected by Gag28-36-specific cytotoxic T cells in HLA-A*2402-positive HIV-1-infected donors, Microbe. Infect. 11 (2009) 198–204.
[36] Y. Yagita, N. Kuse, K. Kuroki, H. Gatanaga, J.M. Carlson, T. Chikata, Z.L. Brumme, H. Murakoshi, T. Akahoshi, N. Pfeifer, S. Mallal, M. John, T. Ose, H. Matsubara, R. Kanda, Y. Fukunaga, K. Honda, Y. Kawashima, Y. Ariumi, S. Oka, K. Maenaka, M. Takiguchi, Distinct HIV-1 escape patterns selected by cytotoxic T cells with identical epitope specificity, J. Virol. 87 (2013) 2253–2263.
[37] H. Sato, Y. Tomita, K. Ebisawa, A. Hachiya, K. Shibamura, T. Shiino, R. Yang, M. Tatsumi, K. Gushi, H. Umeyama, S. Oka, Y. Takebe, Y. Nagai, Augmentation of human immunodeficiency virus type 1 subtype E (CRF01_AE) multiple-drug resistance by insertion of a foreign 11-amino-acid fragment into the reverse transcriptase, J. Virol. 75 (2001) 5604–5613.
[38] A. Adachi, H.E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, M.A. Martin, Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone, J. Virol. 59 (1986) 284–291.
[39] T. Akahoshi, T. Chikata, Y. Tamura, H. Gatanaga, S. Oka, M. Takiguchi, 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 (2012) 1971–1981.
[40] A.C. Karlsson, A.K. Iversen, J.M. Chapman, T. de Oliviera, G. Spotts, A.J. McMichael, M.P. Davenport, F.M. Hecht, D.F. Nixon, Sequential broadening of CTL responses in early HIV-1 infection is associated with viral escape, PLoS One 2 (2007) e225.
[41] W. Kantakamalakul, M. de Souza, S. Bejrachandra, S. Ampol, J. Cox, R. Sutthent, Identification of a novel HIV type 1 CRF01_AE cytotoxic T lymphocyte (CTL) epitope restricted by an HLA-Cw0602 allele and a novel HLA-A0206/peptide restriction, AIDS Res. Hum. Retroviruses 22 (2006) 1271–1282.
[42] F. Li, A.C. Finnefrock, S.A. Dubey, B.T. Korber, J. Szinger, S. Cole, M.J. McElrath, J.W. Shiver, D.R. Casimiro, L. Corey, S.G. Self, Mapping HIV-1 vaccine induced T-cell responses: bias towards less-conserved regions and potential impact on vaccine efficacy in the Step study, PLoS One 6 (2011) e20479.
[43] S. Sabbaj, A. Bansal, G.D. Ritter, C. Perkins, B.H. Edwards, E. Gough, J. Tang, J.J. Szinger, B. Korber, C.M. Wilson, R.A. Kaslow, M.J. Mulligan, P.A. Goepfert, Cross-reactive CD8+ T cell epitopes identified in US adolescent minorities, J. Acquir. Immune Defic. Syndr. 33 (2003) 426–438.
[44] M. Altfeld, M.M. Addo, R.L. Eldridge, X.G. Yu, S. Thomas, A. Khatri, D. Strick, M.N. Phillips, G.B. Cohen, S.A. Islam, S.A. Kalams, C. Brander, P.J. Goulder, E.S. Rosenberg, B.D. Walker, HIV Study Collaboration, Vpr is preferentially targeted by CTL during HIV-1 infection, J. Immunol. 167 (2001) 2743–2752.
[45] M.S. Hossain, H. Tomiyama, T. Inagawa, S. Ida, S. Oka, M. Takiguchi, Identification and characterization of HLA-A*3303-restricted, HIV type 1 Pol- and Gag-derived cytotoxic T cell epitopes, AIDS Res. Hum. Retroviruses 19 (2003) 503–510.
[46] K. Falk, O. Rotzschke, M. Takiguchi, B. Grahovac, V. Gnau, S. Stevanovic, G. Jung, H.G. Rammensee, Peptide motifs of HLA-A1, -A11, -A31, and -A33 molecules, Immunogenetics 40 (1994) 238–241.
[47] Y. Ikeda-Moore, H. Tomiyama, K. Miwa, S. Oka, A. Iwamoto, Y. Kaneko, M. Takiguchi, Identification and characterization of multiple HLA-A24-restricted HIV-1 CTL epitopes: strong epitopes are derived from V regions of HIV-1, J. Immunol. 159 (1997) 6242–6252.
[48] M. Fujiwara, J. Tanuma, H. Koizumi, Y. Kawashima, K. Honda, S. Mastuoka-Aizawa, S. Dohki, S. Oka, M. Takiguchi, Different abilities of escape mutant-specific cytotoxic T cells to suppress replication of escape mutant and wild-type human immunodeficiency virus type 1 in new hosts, J. Virol. 82 (2008) 138–147.
[49] C. Brander, O.O. Yang, N.G. Jones, Y. Lee, P. Goulder, R.P. Johnson, A. Trocha, D. Colbert, C. Hay, S. Buchbinder, C.C. Bergmann, H.J. Zweerink, S. Wolinsky, W.A. Blattner, S.A. Kalams, B.D. Walker, Efficient processing of the immunodominant, HLA-A*0201-restricted human immunodeficiency virus type 1 cytotoxic T-lymphocyte epitope despite multiple variations in the epitope flanking sequences, J. Virol. 73 (1999) 10191–10198.
[50] S. Buranapraditkun, U. Hempel, P. Pitakpolrat, R.L. Allgaier, P. Thantivorasit, S.I. Lorenzen, S. Sirivichayakul, W.H. Hildebrand, M. Altfeld, C. Brander, B.D. Walker, P. Phanuphak, P. Hansasuta, S.L. Rowland-Jones, T.M. Allen, K. Ruxrungtham, A novel immunodominant CD8+ T cell response restricted by a common HLA-C allele targets a conserved region of Gag HIV-1 clade CRF01_AE infected Thais, PLoS One 6 (2011) e23603.

High Prevalence of Illicit Drug Use in Men Who Have Sex with Men with HIV-1 Infection in Japan

Takeshi Nishijima[1,2], Hiroyuki Gatanaga[1,2]*, Hirokazu Komatsu[3], Misao Takano[1], Miwa Ogane[1], Kazuko Ikeda[1], Shinichi Oka[1,2]

1 AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, **2** Center for AIDS Research, Kumamoto University, Kumamoto, Japan, **3** Department of Community Care, Saku Central Hospital, Nagano, Japan

Abstract

Objective: To examine the prevalence of illicit drug use among men who have sex with men (MSM) with HIV-1 infection in Japan, where the life-time prevalence of illicit drug use in the general population is only 2.9%.

Design: A single-center cross-sectional study at a large HIV clinic in Tokyo, which treats approximately 15% of HIV-1 infected patients in Japan.

Methods: The prevalence of illicit drug use and the assciation of characteristics and social demographics of the patients with illicit drug use were examined. Patients who visited the clinic for the first time from 2005 to 2010 were enrolled. Relevant variables were collected using a structured interview and from the medical records. Multivariate logistic regression analyses were applied to estimate the odds of association of MSM over non-MSM HIV-infected patients with illicit drug use.

Results: 1,196 patients were enrolled. They were mostly Japanese men of relatively young age. Illicit drug use (including injection drugs) was reported by 35% of the patients (by 40% of MSM), and 4% were IDU while 5% were on methamphetamine. 2% of the population was arrested due to illicit drugs. MSM was significantly associated with illicit drug use (adjusted OR = 4.60; 95% CI, 2.88–7.36; $p<0.01$). Subgroup analysis of the patients stratified by three age groups (≤30, 31 to 40, and >40) showed that the odds of association of MSM with illicit drug use was the strongest in the youngest age group (≤30 years: adjusted OR = 7.56; 95% CI, 2.86–20.0; $p<0.01$), followed by the oldest (>40 years: adjusted OR = 6.15; 95% CI, 2.40–15.8; $p<0.01$), and the weakest in the group aged 31 to 40 (adjusted OR = 3.39; 95% CI, 1.73–6.63; $p<0.01$).

Conclusions: The prevalence of illicit drug use is high among MSM patients with HIV-1 infection in Japan. Effective intervention for illicit drug use in this population is warranted.

Citation: Nishijima T, Gatanaga H, Komatsu H, Takano M, Ogane M, et al. (2013) High Prevalence of Illicit Drug Use in Men Who Have Sex with Men with HIV-1 Infection in Japan. PLoS ONE 8(12): e81960. doi:10.1371/journal.pone.0081960

Editor: Wenzhe Ho, Temple University School of Medicine, United States of America

Received May 8, 2013; **Accepted** October 18, 2013; **Published** December 10, 2013

Funding: This work was supported by Grants-in Aid for AIDS research from the Japanese Ministry of Health, Labour, and Welfare (H23-AIDS-001). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: higatana@acc.ncgm.go.jp

Introduction

Illicit drug users, especially injection drug users (IDU), are at high risk of infection with HIV-1 [1,2]. They are one of the "difficult to reach" populations, especially with regard obtaining accurate prevalence data [3]. In Japan, the prevalence of illicit drug use in the general population is only 2.9% according to the 2009 Nationwide General Population Survey on Drug Use and Abuse [4,5] (http://www.ncnp.go.jp/nimh/pdf/h21.pdf. in Japanese) (http://www.mhlw.go.jp/bunya/iyakuhin/yakubuturanyou/torikumi/dl/index-04.pdf. in Japanese). To our knowledge, however, no study has examined the prevalence of illicit drug use among patients with HIV-1 infection in Japan.

Among patients with HIV-1 infection, illicit drug use is associated with lower antiretroviral therapy (ART) uptake and inferior adherence [6–9], which leads to suboptimal treatment outcome, compared with patients with other risk categories [10–12]. The aim of the present study was to examine the prevalence of illicit drug use in patients with HIV-1 infection and its association with characteristics of the patients in Japan, in order to establish effective intervention strategies.

Methods

Ethics Statement

This study was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine, Tokyo, Japan. The Committee waived a written informed consent, because this study only used data of patients from routine clinical practice. However, at our clinic each patient provided a written informed consent for the clinical and laboratory data to be used and published for research purposes [13]. We conducted this study according to the principles expressed in the Declaration of Helsinki.

Study design

This study was designed and reported according to the recommendations of Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement [14]. We performed a single center cross-sectional study of patients with HIV-1 infection to examine the prevalence of illicit drug according to patient characteristics including sexual orientation, primarily focusing on men who have sex with men (MSM). Illicit drugs were defined as legally prohibited substances in Japan; They included amyl nitrite and 5-methoxy-diisopropyltryptamine, which became prohibited by law in 2006 and 2005, respectively, in Japan [15]. This study was conducted at the AIDS Clinical Center, Tokyo. Our facility is one of the largest clinics for HIV care in Japan with more than 3,300 registered patients [13]. Considering that the total reported number of patients with HIV-1 infection is 21,415 by the end of 2011, this clinic treats approximately 15% of the HIV-1 infected patients in Japan (http://api-net.jfap.or.jp/status/2011/11nenpo/hyo_02.pdf. in Japanese).

Study Subjects

The study population comprised patients with HIV-1 infection, aged >17 years, who visited our clinic for the first time from January 1, 2005 to August 31, 2010. The following exclusion criteria were applied; 1) those who visited the clinic for a second opinion, 2) those referred to other facilities on their first or second visit. These patients were excluded because the structured interview on social demographics was often not conducted in these patients, 3) patients infected through contaminated blood products (e.g. hemophiliacs) and mother to child transmission, and 4) patients who refused to be included in the study.

Measurements

Variables were collected through a structured interview conducted at the first visit as part of routine clinical practice by the nurses specializing at the HIV outpatient care. The interview by these "coordinator nurses" included the following variables: history of illicit drug use and injection drug use (and their types if available), perceived route of transmission, sexual orientation (men were asked whether they have sex with men), history of gay bathhouse use (if MSM), working status, and living status (alone or with someone else) [16]. Because interviews could potentially underestimate the prevalence of illicit drug use, we also searched the medical records for information on illicit drug use and related variables covering the period from the first visit to December 2012. Data of age, sex, ethnicity, current treatment status for HIV infection, and history of AIDS (defined as history of or concurrent 23 AIDS-defining diseases set by the Japanese Ministry of Health, Labour and Welfare) were obtained from the medical records (http://www.haart-support.jp/pdf/guideline2012.pdf in Japanese). The laboratory data of CD4 cell count, HIV-1 viral load, hepatitis C antibody on the first visit were also collected, and when these tests were not conducted on that day, data within three months from the first visit were used.

Statistical analysis

Patients' characteristics and social demographics were compared between MSM and non-MSM groups by the Student's t-test for continuous variables and by either the χ^2 test or Fisher's exact test for categorical variables. Logistic regression analysis was used to estimate the odds of association of MSM, relative to non-MSM, with illicit drug use. The odds of association of each basic demographics, baseline laboratory data, and other medical conditions listed above was also estimated with univariate analysis.

To estimate the odds of association of MSM over non-MSM with illicit drug use, we conducted multivariate logistic regression analysis adjusted by age and ethnicity. Age and ethnicity (Japanese) were selected among four variables with p value <0.05 in univariate analysis, because age is a basic demographic and the literature had reported that population/ethnicity can affect the prevalence of illicit drug use [17]. The two variables; "ART" and "history of AIDS" were not included because they were not considered to be related to illicit drug use.

To estimate the odds of association of different age categories with illicit drug use, we divided the group into three age subgroups: ≤30, 31 to 40, and >40 years. Then, the above-mentioned multivariate analysis was conducted for each subgroup.

Statistical significance was defined at two-sided p value of <0.05. We used odds ratios (ORs) and 95% confidence intervals (95% CIs) to estimate the odds of association of each variable with illicit drug use. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 20.0 (SPSS, Chicago, IL).

Results

During the study period, 1,366 patients with HIV-1 infection visited the AIDS Clinical Center for the first time, and 170 patients were excluded from the analysis based on the above-mentioned exclusion criteria (Figure 1). For the 1,196 patients included in the study, the perceived route of transmission was male-to-male sexual contact in 948 (79%), heterosexual contact in 173 (14%), IDU in 22 (2%), and unknown in 53 (4%). The majority of the study patients were relatively young Japanese men with a median age of 36 years. Most patients were ART-naïve, with a median CD4 count of 245/μl (Table 1).

Among the 1,196 patients, 415 (35%) had used or were illicit drug users, and 53 (4%) were IDUs while 63 (5%) reported using methamphetamine. With regard to social history, 27 (2%) had been detained or arrested due to possession or use of illicit drugs (Table 1). Among the illicit drugs used, amyl nitrite and 5-methoxy-diisopropyltryptamine were the most commonly named by the patients. 3,4-methylenedioxymethamphetamine, cannabis, heroin, cocaine, and opium were also mentioned (numbers not counted except for methamphetamine).

Of the 1,196 patients, 973 (81%) were MSM regardless of the perceived route of transmission (e.g., if a patient considered to have been infected with HIV-1 through injection drug use and was MSM, he was classified as MSM in Table 1). Compared with non-MSM patients, MSM were significantly younger and more likely to be Japanese. MSM patients were more likely to have experienced illicit drugs [392 (40%)] than non-MSM [23 (10%), p<0.01], and have used methamphetamine [57 (6%) versus 6 (3%), p = 0.07], and to have been arrested/detained due to illicit drug use/possession [(26 (3%) versus 1 (0.4%), p = 0.04) (Table 1). There was no difference in the percentage of IDUs among the MSM and non-MSM groups [44 (5%) versus 9 (4%), p = 0.73]. The CD4 count of MSM patients tended to be higher, and MSM were less likely to present with AIDS than non-MSM, although HIV viral load of MSM was significantly higher than that of non-MSM. MSM were more likely to have a job and be living alone. Further analysis showed that 47% of MSM patients used a gay bathhouse, and among them, the prevalence of illicit drug use was higher (49%) than all MSM (40%). The prevalence of illicit drug use was even higher in MSM aged ≤30 years (52%).

Univariate analysis showed a significant relationship between MSM and illicit drug use (OR = 5.87; 95% CI, 3.74–9.20; p<0.01) (Table 2, Model 1). Furthermore, younger age, being

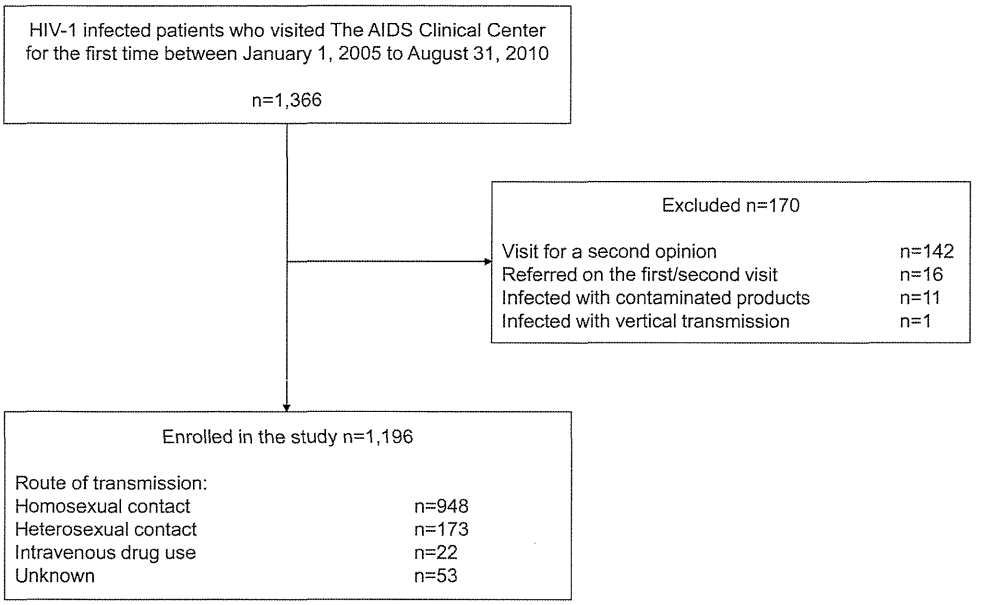

Figure 1. Patient enrollment.
doi:10.1371/journal.pone.0081960.g001

Table 1. Baseline characteristics of total study patients, MSM, and non-MSM.

	Total (n = 1,196)	MSM (n = 973)	Non-MSM (n = 223)	P value
Sex (male), n (%)	1,114 (93)	973 (100)	152 (63)	<0.01
Age (years)[†]	36 (29–43)	35 (29–42)	38 (31–47)	<0.01
History of illicit drug use, n (%)	415 (35)	392 (40)	23 (10)	<0.01
Injection drug use, n (%)	53 (4)	44 (5)	9 (4)	0.73
Methamphetamine use, n (%)	63 (5)	57 (6)	6 (3)	0.07
Arrested due to illicit drugs, n (%)	27 (2)	26 (3)	1 (0.4)	0.04
History of gay bathhouse use, n (%)	Not applicable	461 (47)		
Ethnicity, n (%)[a]				
Japanese	1058 (89)	906 (93)	152 (68)	<0.01
Asian	70 (6)	29 (3)	41 (18)	
White	27 (2)	26 (3)	1 (0.4)	
Black	26 (2)	2 (0.2)	24 (11)	
Latino	12 (1)	7 (1)	5 (2)	
Working status, n (%)[b]				
Without job	226 (19)	163 (17)	63 (28)	<0.01
Working	902 (75)	763 (78)	139 (63)	
Student	56 (5)	47 (5)	9 (4)	
Housewife	11 (1)	0	11 (5)	
Living alone, n (%)[c]	530 (44)	475 (49)	55 (25)	<0.01
CD4 count (/μl)[†d]	245 (101–379)	252 (114–380)	207 (50–379)	0.08
HIV-1 viral load (log_{10}/ml)[†e]	4.60 (3.91–5.20)	4.64 (3.94–5.20)	4.43 (3.26–5.08)	<0.01
On antiretroviral therapy, n (%)	120 (10)	85 (9)	35 (16)	<0.01
History of AIDS, n (%)	321 (27)	247 (25)	74 (33)	0.02
Positive HCV antibody, n (%)	38 (3)	19 (2)	19 (9)	<0.01

[†]median (interquartile range).
Data of [a]three, [b]one, [c]fifteen, [d]two, and [e]four patients were not available (missing).
doi:10.1371/journal.pone.0081960.t001

Japanese, on ART, and history of AIDS were associated with illicit drug use. On the other hand, without a job, living alone, and positive HCV antibody were not associated with illicit drug use. Multivariate analysis identified MSM to be significantly associated with illicit drug use after adjustment for age and Japanese (adjusted OR = 4.60; 95% CI, 2.88–7.36; p<0.01) (Table 2, Model 2).

Subgroup analysis of the patients stratified by three age groups (≤30, 31 to 40, and >40) showed that the odds of association of MSM with illicit drug use was the strongest in the youngest age group (≤30 years: adjusted OR = 7.56; 95% CI, 2.86–20.0; p<0.01), followed by the oldest (>40 years: adjusted OR = 6.15; 95% CI, 2.40–15.8; p<0.01), and the weakest in the group aged 31 to 40 (adjusted OR = 3.39; 95% CI, 1.73–6.63; p<0.01) (Table 3).

Table 3. Results of multivariate analysis of the association of MSM over non-MSM for illicit drug use according to age.

	Adjusted OR	95% CI	P value
Age ≤30 years (n = 369)			
MSM vs. non-MSM	7.56	2.86–20.0	<0.01
Age 31 to 40 years (n = 473)			
MSM vs. non-MSM	3.39	1.73–6.63 85	<0.01
Age >40 years (n = 354)			
MSM vs. non-MSM	6.15	2.40–15.8	<0.01

MSM was adjusted with the same variables as Model 2, Table 2.
MSM: men who have sex with men.
doi:10.1371/journal.pone.0081960.t003

Discussion

The prevalence of illicit drug use among patients with HIV-1 infection in this large urban HIV clinic in Tokyo, which treats approximately 15% of patients with HIV-1 infection in Japan, was high at 35%. The prevalence was higher among HIV-1 infected MSM (40%), especially among young MSM aged ≤30 years (52%). Furthermore, HIV-1 infected MSM were more likely to use methamphetamine and to be arrested due to illicit drugs, compared with non-MSM. It should be emphasized that these numbers are likely to be underreported, since some patients would not admit illicit drug use to the interviewers on their first visit.

To our knowledge, this is the first study on the prevalence of illicit drug use among patients with HIV-1 infection in Japan. Although the prevalence of illicit drug use is considered extremely low among the general population in Japan with lifetime prevalence of 2.9% in 2009, high prevalence of illicit drug use in patients with HIV-1 infection, especially among HIV-1 infected MSM, was demonstrated [4,5] (http://www.ncnp.go.jp/nimh/pdf/h21.pdf. in Japanese) (http://www.mhlw.go.jp/bunya/iyakuhin/yakubuturanyou/torikumi/dl/index-04.pdf. in Japanese). The prevalence of methamphetamine use and incarceration due to illicit drug was also high, suggesting a substantial impact of illicit drugs, not only on the well-being of this population in terms of both medical and social perspectives, but also on public health perspectives [11,12].

In Japan, the number of illicit drug users arrested in 2010 was 14,965. Among these, 12,200 used methamphetamine, followed by cannabis (2,367), while only several hundred at most used other drugs (http://www.mhlw.go.jp/bunya/iyakuhin/yakubuturanyou/torikumi/dl/index-01.pdf in Japanese). Of note, the number of arrestees due to other injectable drugs, such as heroin and cocaine, was small (22 and 112, respectively). Thus, most injection drug users in Japan are methamphetamine users. Majority of the patients identified as IDU in this study were considered to be methamphetamine users as well.

By the end of 2011, of 19,976 patients (excluding those infected with contaminated blood products) reported to be infected with HIV-1, 108 (0.5%) were reported to be infected through injection drug use according to the surveillance conducted by the AIDS Surveillance Committee of the Japanese Ministry of Health, Labour and Welfare (http://api-net.jfap.or.jp/status/2011/11nenpo/hyo_02.pdf in Japanese). The prevalence of IDUs in this study is substantially higher; 53 (4%) of the 1,196 were IDUs, suggesting a considerable underreporting of IDU in the surveillance data. It is well known that for IDUs, prognosis is much worse than non-injecting drug users, as one multicenter study conducted in Europe and North America reported that IDUs experienced approximately five times higher mortality rates than patients infected through sexual intercourse [18]. Although the prevalence of IDUs among patients with HIV-1 infection in Japan is still much lower than that in neighboring countries, such as Taiwan (27.6%) and China (24.3%), there is an urgent need to develop effective prevention programs for HIV-1 infected illicit drug users [19] (http://www.unaids.org.cn/download/2009%20China%20Estimation%20Report-En.pdf) (http://www.cdc.gov.tw/english/list.aspx?treeid = 00ED75D6C887BB27&nowtreeid = 334C2073091C8677).

Although the prognosis of injection drug users is reported to be worse than that of non-injection drug users [20], this study primarily focused on illicit drug use as a whole, rather than injection drug use. This is because only a few studies focused on illicit drug use among HIV-1 infected patients, although a large number of studies focused on injection drugs [21–25]. Illicit drug use in patients with HIV-1 infection is an important issue, because not only illicit drug use lead to inferior treatment outcome compared with non users [10–12], but also non injection drug users are prone to practice high risk sexual behaviors, which might lead to transmission of HIV and other infectious diseases [8,26]. Studies from the US reported that especially MSM who use illicit

Table 2. Results of multivariate analysis of the association of MSM over non-MSM for illicit drug use.

	Model 1 Crude n = 1,196		Model 2 Adjusted n = 1,196	
	OR	95% CI	OR	95% CI
Men who have sex with men†	5.87	3.74–9.20	4.60	2.88–7.36
Age per 1 year†			0.95	0.94–0.97
Japanese†			1.74	1.07–2.82

†p<0.05.
doi:10.1371/journal.pone.0081960.t002

drugs are at high risk for HIV and sexual transmitted infections due to close associations between risky sexual behaviors and illicit drug use [27,28] Furthermore, illicit drug use, especially opioid use, can be a trajectory into injection drug use [29,30].

Several limitations need to be acknowledged. First, due to the nature of single-center study, this is a convenience sample and the results of this study do not necessarily represent the prevalence of illicit drug use in all patients with HIV-1 infection in Japan. However, as mentioned above, our clinic treats approximately 15% of the total HIV patients in Japan, and furthermore, most HIV-1 infected patients reside in urban areas such as Tokyo metropolitan area (http://api-net.jfap.or.jp/status/2011/11nenpo/hyo_02.pdf in Japanese). Thus, the discrepancy in the prevalence of illicit drug use between the study patients and all HIV patients in Japan should not be too large. Second, the structured interview method to collect data cannot avoid underreporting of illicit drug usage. Thus, the prevalence of illicit drug use in this population is very likely to be higher than what is reported here. However, underreporting to a certain degree is unavoidable with regard to issues such as illicit drug use [3].

In conclusion, the prevalence of illicit drug use in patients with HIV-1 infection in this large HIV clinic in Tokyo was high at 35%, and was higher in HIV-1 infected MSM (40%). Despite the low prevalence of IDUs (0.5%) among HIV-infected patients reported by the AIDS Surveillance Committee, 5% of patients in this study were IDUs. All relevant parties to the issue of illicit drug use in patients with HIV-1 infection need to recognize that illicit drug use is a huge burden in care and well-being of this population even in Japan, a country with very low prevalence of illicit drug use in the general population. Appropriate measures for prevention and intervention of illicit drug use are urgently needed to ensure proper treatment and prevention of spread of HIV infection.

Acknowledgments

The authors thank Dr. Kiyoshi Wada, Department of Drug Dependence Research, National Institute of Mental Health, National Center of Neurology and Psychiatry, and Dr. Keishiro Yajima, AIDS Medical Center, National Hospital Organization Osaka National Hospital, for valuable comments for the manuscript. The authors also thank "coordinator nurses" who conducted the structured interviews (Ruiko Yakuwa, Beni Ito, Yuko Sugino, Miki Koyama, Kenji Takeda, Megumi Shimada, Jongmi Seo, Yuki Yamada, Kyoko Ishigaki), and all other clinical staff at the AIDS Clinical Center, for their help in the completion of this study.

Author Contributions

Conceived and designed the experiments: TN HG HK MT SO. Performed the experiments: MO KI. Analyzed the data: TN HK HG MT SO. Contributed reagents/materials/analysis tools: MO KI SO. Wrote the paper: TN HG MT SO.

References

1. Lehman JS, Allen DM, Green TA, Onorato IM (1994) HIV infection among non-injecting drug users entering drug treatment, United States, 1989–1992. Field Services Branch. AIDS 8: 1465–1469.
2. Hahn RA, Onorato IM, Jones TS, Dougherty J (1989) Prevalence of HIV infection among intravenous drug users in the United States. JAMA 261: 2677–2684.
3. Magnani R, Sabin K, Saidel T, Heckathorn D (2005) Review of sampling hard-to-reach and hidden populations for HIV surveillance. AIDS 19 Suppl 2: S67–72.
4. Wada K (2011) The history and current state of drug abuse in Japan. Ann N Y Acad Sci 1216: 62–72.
5. Tominaga M, Kawakami N, Ono Y, Nakane Y, Nakamura Y, et al. (2009) Prevalence and correlates of illicit and non-medical use of psychotropic drugs in Japan: findings from the World Mental Health Japan Survey 2002–2004. Soc Psychiatry Psychiatr Epidemiol 44: 777–783.
6. Wood E, Montaner JS, Tyndall MW, Schechter MT, O'Shaughnessy MV, et al. (2003) Prevalence and correlates of untreated human immunodeficiency virus type 1 infection among persons who have died in the era of modern antiretroviral therapy. J Infect Dis 188: 1164–1170.
7. Strathdee SA, Palepu A, Cornelisse PG, Yip B, O'Shaughnessy MV, et al. (1998) Barriers to use of free antiretroviral therapy in injection drug users. JAMA 280: 547–549.
8. Malta M, Magnanini MM, Strathdee SA, Bastos FI (2010) Adherence to antiretroviral therapy among HIV-infected drug users: a meta-analysis. AIDS Behav 14: 731–747.
9. Horstmann E, Brown J, Islam F, Buck J, Agins BD (2010) Retaining HIV-infected patients in care: Where are we? Where do we go from here? Clin Infect Dis 50: 752–761.
10. Weber R, Huber M, Rickenbach M, Furrer H, Elzi L, et al. (2009) Uptake of and virological response to antiretroviral therapy among HIV-infected former and current injecting drug users and persons in an opiate substitution treatment programme: the Swiss HIV Cohort Study. HIV Med 10: 407–416.
11. Milloy MJ, Marshall BD, Kerr T, Buxton J, Rhodes T, et al. (2012) Social and structural factors associated with HIV disease progression among illicit drug users: a systematic review. AIDS 26: 1049–1063.
12. Porter K, Babiker A, Bhaskaran K, Darbyshire J, Pezzotti P, et al. (2003) Determinants of survival following HIV-1 seroconversion after the introduction of HAART. Lancet 362: 1267–1274.
13. Nishijima T, Gatanaga H, Komatsu H, Tsukada K, Shimbo T, et al. (2012) Renal function declines more in tenofovir- than abacavir-based antiretroviral therapy in low-body weight treatment-naive patients with HIV infection. PLoS One 7: e29977.
14. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, et al. (2007) Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Epidemiology 18: 805–835.
15. Hidaka Y, Ichikawa S, Koyano J, Urao M, Yasuo T, et al. (2006) Substance use and sexual behaviours of Japanese men who have sex with men: a nationwide internet survey conducted in Japan. BMC Public Health 6: 239.
16. Nishijima T, Gatanaga H, Komatsu H, Takano M, Ogane M, et al. (2013) Illicit Drug Use Is a Significant Risk Factor for Loss to Follow Up in Patients with HIV-1 Infection at a Large Urban HIV Clinic in Tokyo. PLoS One 8: e72310.
17. Millett GA, Peterson JL, Wolitski RJ, Stall R (2006) Greater risk for HIV infection of black men who have sex with men: a critical literature review. Am J Public Health 96: 1007–1019.
18. Zwahlen M, Harris R, May M, Hogg R, Costagliola D, et al. (2009) Mortality of HIV-infected patients starting potent antiretroviral therapy: comparison with the general population in nine industrialized countries. Int J Epidemiol 38: 1624–1633.
19. Chen YM, Kuo SH (2007) HIV-1 in Taiwan. Lancet 369: 623–625.
20. Qian HZ, Stinnette SE, Rebeiro PF, Kipp AM, Shepherd BE, et al. (2011) The relationship between injection and noninjection drug use and HIV disease progression. J Subst Abuse Treat 41: 14–20.
21. Giordano TP, Hartman C, Gifford AL, Backus LI, Morgan RO (2009) Predictors of retention in HIV care among a national cohort of US veterans. HIV Clin Trials 10: 299–305.
22. Rice BD, Delpech VC, Chadborn TR, Elford J (2011) Loss to follow-up among adults attending human immunodeficiency virus services in England, Wales, and Northern Ireland. Sex Transm Dis 38: 685–690.
23. Ndiaye B, Ould-Kaci K, Salleron J, Bataille P, Bonnevie F, et al. (2009) Incidence rate and risk factors for loss to follow-up in HIV-infected patients from five French clinical centres in Northern France - January 1997 to December 2006. Antivir Ther 14: 567–575.
24. Mocroft A, Kirk O, Aldins P, Chies A, Blaxhult A, et al. (2008) Loss to follow-up in an international, multicentre observational study. HIV Med 9: 261–269.
25. Lebouche B, Yazdanpanah Y, Gerard Y, Sissoko D, Ajana F, et al. (2006) Incidence rate and risk factors for loss to follow-up in a French clinical cohort of HIV-infected patients from January 1985 to January 1998. HIV Med 7: 140–145.
26. Latkin CA, Knowlton AR, Sherman S (2001) Routes of drug administration, differential affiliation, and lifestyle stability among cocaine and opiate users: implications to HIV prevention. J Subst Abuse 13: 89–102.
27. Forrest DW, Metsch LR, LaLota M, Cardenas G, Beck DW, et al. (2010) Crystal methamphetamine use and sexual risk behaviors among HIV-positive and HIV-negative men who have sex with men in South Florida. J Urban Health 87: 480–485.
28. Mansergh G, Shouse RL, Marks G, Guzman R, Rader M, et al. (2006) Methamphetamine and sildenafil (Viagra) use are linked to unprotected receptive and insertive anal sex, respectively, in a sample of men who have sex with men. Sex Transm Infect 82: 131–134.

29. Lankenau SE, Teti M, Silva K, Jackson Bloom J, Harocopos A, et al. (2012) Initiation into prescription opioid misuse amongst young injection drug users. Int J Drug Policy 23: 37–44.

30. Pollini RA, Banta-Green CJ, Cuevas-Mota J, Metzner M, Teshale E, et al. (2011) Problematic use of prescription-type opioids prior to heroin use among young heroin injectors. Subst Abuse Rehabil 2: 173–180.

WHO Antiretroviral Therapy Guidelines 2010 and Impact of Tenofovir on Chronic Kidney Disease in Vietnamese HIV-Infected Patients

Daisuke Mizushima[1,2*], Junko Tanuma[1], Fumihide Kanaya[1], Takeshi Nishijima[1,2], Hiroyuki Gatanaga[1,2], Nguyen Tien Lam[3], Nguyen Thi Hoai Dung[3], Nguyen Van Kinh[3], Yoshimi Kikuchi[1], Shinichi Oka[1,2*]

1 AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, **2** Center for AIDS Research, Kumamoto University, Kumamoto, Japan, **3** National Hospital of Tropical Diseases, Hanoi, Vietnam

Abstract

Objective: The 2010 WHO antiretroviral therapy (ART) guidelines have resulted in increased tenofovir use. Little is known about tenofovir-induced chronic kidney disease (CKD) in HIV-infected Vietnamese with mean body weight of 55 kg. We evaluated the prevalence and risk factors of CKD in this country.

Design: Cross-sectional study was performed.

Methods: Clinical data on HIV-infected Vietnamese cohort were collected twice a year. To evaluate the prevalence of CKD, serum creatinine was measured in 771 patients in October 2011 and April 2012. CKD was defined as creatinine clearance less than 60 ml/min at both time points. Multivariate logistic regression was used to determine the factors associated with CKD

Results: Tenofovir use increased in Vietnam from 11.9% in April 2011 to 40.3% in April 2012. CKD was diagnosed in 7.3%, of which 7% was considered moderate and 0.3% was severe. Multivariate analysis of October-2011 data identified age per year-increase (OR: 1.229, 95%CI, 1.170-1.291), body weight per 1 kg-decrement (1.286, 1.193-1.386), and tenofovir use (2.715, 1.028-7.168) as risk factors for CKD.

Conclusions: Older age, low body weight and tenofovir use were independent risk factors for CKD in Vietnam. Further longitudinal study is required to evaluate the impact of TDF on renal function in Vietnam and other countries with small-body weight patients.

Citation: Mizushima D, Tanuma J, Kanaya F, Nishijima T, Gatanaga H, et al. (2013) WHO Antiretroviral Therapy Guidelines 2010 and Impact of Tenofovir on Chronic Kidney Disease in Vietnamese HIV-Infected Patients . PLoS ONE 8(11): e79885. doi:10.1371/journal.pone.0079885

Editor: Michael Alan Polis, National Institute of Allergy and Infectious Diseases, United States of America

Received June 11, 2013; **Accepted** September 25, 2013; **Published** November 6, 2013

Funding: This study was supported by Japan Initiative for Global Research Network on Infectious Diseases (J-GRID). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

* E-mail: oka@acc.ncgm.go.jp (SO); dmizushi@acc.ncgm.go.jp (DM)

Introduction

Advances in antiretroviral therapy (ART) had turned HIV/AIDS into a chronic□disease [1-5]. As a consequence of living longer, chronic kidney disease (CKD) has become an important cause of morbidity and mortality in HIV-infected patients [1,3-5]. Several studies have reported increased prevalence of CKD, ranging from 4.9% to 8.4% in such patients [6-9]. In addition to the established risk factors, such as aging, diabetes mellitus (DM) and hypertension [2,10], other factors related to the virus itself and to the treatment [e.g., exposure to tenofovir (TDF), a commonly used antiretroviral (ARV)], are thought to be related to nephrotoxicity in HIV-infected patients[2,11,12].

To date, the benefit of TDF first line treatment is considered to outweigh the risk of TDF-induced nephrotoxicity. A recent meta-analysis study has reported that the use of TDF is associated with a statistically significant though only modest renal dysfunction, and recommended no restriction of TDF use when regular monitoring of renal function and serum phosphate levels is impractical [13]. Furthermore, the 2010 WHO guidelines for ART in adults and adolescents recommended TDF as part of the first line regimens (URL: http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf).

However, several studies have reported that low body weight is an independent risk factor for TDF-associated nephrotoxicity and might lead to potentially higher risk for larger drug exposure and thus, more severe toxicity [14-17]. Under such

scenario, regional prevalence of CKD may influence the approach to screening and monitoring of HIV-infected patients initiated on ART. In particular, most nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), such as TDF and 3TC/FTC, are excreted by the kidney and may require dose adjustment in individuals with reduced glomerular filtration rate (GFR), and may require more intensive monitoring in patients with pre-existing CKD.

Following the 2010 WHO guidelines, the use of TDF has been increasing rapidly in Vietnam, where patients are more likely to have smaller body weight compared to Caucasians. At this stage, little is known about CKD among Vietnamese HIV-infected patients. In this context, it is important to determine the prevalence of CKD and its risk factors including TDF exposure and low body weight in this region. The present study was conducted to evaluate the above factors in Vietnamese HIV-infected patients.

Methods

Study design

We performed a cross-sectional study with an observational single-center cohort of Vietnamese HIV-infected patients on ART. This cohort was established since 2007 at the National Hospital of Tropical Disease in Hanoi, one of the largest outpatient clinics for HIV infected-patients in Vietnam. Clinical data are collected twice a year (in April and October) in this cohort. The population of this cohort comprised HIV-infected patients on ART aged more than 17 years. To evaluate CKD in this group, serum creatinine had been examined since October 2011. Serum creatinine was measured in October 2011 and April 2012. Patients whose creatinine was not obtained at both time points were excluded from the study. Other clinical data were collected twice a year (in April and October) as well. The study was approved by the Human Research Ethics Committee of National Hospital of Tropical Disease and Hanoi city. Each patient included in this study provided a written informed consent for the clinical and laboratory data to be used for publication. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Measurements

Data included demographic variables (height, weight, sex and age); a complete history of ART; use of cotrimoxazole; CD4 cell count (cell/mm^3, measured by flow cytometry); plasma HIV-RNA (copies/ml, measured by the Roche COBAS TaqMan HIV monitor assay); serum creatinine (mg/dl, measured by Jaffe method); date of HIV diagnosis and other comorbidities. CKD was defined as creatinine clearance (Ccl) estimated by the Cockcroft-Gault formula of <60 ml/min at October 2011 and April 2012 (6 months apart). Renal dysfunction at each time point was also classified into five stages according to the guidelines of the National Kidney Foundation [18]: normal renal function: Ccl ≥90 ml/min; mild renal dysfunction, Ccl between 60-89 ml/min; moderate, Ccl 30-59 ml/min; severe renal dysfunction, Ccl 15-29 ml/min; and renal failure or dialysis, with Ccl of <15 ml/min.

Statistical analysis

Statistical analysis included descriptive (mean and standard deviation), univariate and multivariate analyses. Absolute and relative frequencies were utilized for continuous and categorical variables, respectively. To evaluate the association between CKD and categorical variables, the chi-square test or Fisher exact test was applied as required. Independent T test or one-way analysis of variance (ANOVA) was used to compare mean values of normally distributed data and the Mann Whitney test or Kruskal-Wallis test for parameters with skewed data distribution. Variables significantly associated with renal dysfunction in univariate analysis ($p<0.05$) were entered into multivariate analysis. Logistic regression was used to determine the factors associated with CKD in univariate and multivariate analyses. Statistical significance was defined at two-sided p value <0.05. We used the odds ratio (OR) and 95% confidence interval (95% CI) to estimate the association of each variable with renal dysfunction. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Patients on TDF

The percentage of TDF use in our cohort increased from 11.9% in April 2011 to 40.3% in April 2012. In contrast, stavudine (d4T) use decreased from 37.8% in April 2011 to 14.6% in April 2012. The patterns of use of TDF and d4T well reflected the recommendation of the 2010 WHO ART guidelines; recommendation for the use of TDF or zidovudine (AZT) and phasing out of d4T.

Prevalence of CKD and renal dysfunction at each time point

To determine the prevalence of CKD, serum creatinine was measured in 771 patients in October 2011 and April 2012. CKD was diagnosed in 56 (7.3 %) patients and classified as moderate in 54 and severe in 2 (Table **1**). The number of patients with moderate and severe renal dysfunction increased from 74 (9.6%) in October 2011 to 111 (14.4%) in April 2012. The data of serum creatinine by CKD stage are shown in Table **1**.

Baseline demographics and laboratory data

Table **2** compares the baseline demographics and clinical variables of patients with or without CKD for the data of October 2011. Patients with CKD were significantly older, more likely to be diabetic females treated with TDF and lopinavir boosted with ritonavir, and of significantly lower body weight with higher serum creatinine, and with history of AIDS-defining disease, compared to those without CKD. CD4 count, HIV RNA viral load, and duration of ART were not significantly different between the two groups. The mean CD4 count was >300/mm^3 and the mean HIV RNA load was <100 copies/ml in both groups.

Table 1. Prevalence of CKD and renal function at two time points in 771 HIV-infected Vietnamese on ART.

		CKD	Oct 2011	Apr 2012
Renal function	Ccl (ml/min)		n (%)	
Normal	90 or more	-	178 (23.0)	159 (20.6)
Mild reduction	60-89	-	519 (67.4)	501 (65.0)
Moderate reduction	30-59	54 (7.0)	72 (9.3)	108 (14.0)
Severe reduction	15-29	2 (0.3)	2 (0.3)	3 (0.4)
Renal failure	less than 15	0	0	0

Renal dysfunction was classified according to the guidelines of the National Kidney Foundation (18)

CKD was defined as Ccls of <60 ml/min at both time points (October 2011 and April 2012).

CKD; chronic kidney disease, ART; antiretroviral therapy

doi: 10.1371/journal.pone.0079885.t001

Table 2. Baseline demographics and laboratory data of 771 patients measured at October 2011.

variables	Entire group	CKD (+)	CKD(-)	P value
Number of patients	771	56 (7.3%)	715 (92.7%)	
Age, years	36.4±7. 86	46.5±11.5	35.6±6.9	<0.001
Female, n (%)	296 (38.4%)	36 (64.3)	260 (36.4)	<0.001
Body weight, kg	55.0±8.4	47.1±6.3	55.6±8.2	<0.001
Diabetes mellitus, n (%)	32 (4.2%)	6 (10.7)	26 (3.6)	0.023
Serum creatinine, mg/dl	0.95±0. 15	1.11±0.22	0.94±0.13	<0.001
CD4+ count, /μl	349.0±202.8	337.0±215.2	349.9±201.9	0.648
HIV RNA, log_{10} c/ml	1.79±0.52	1.80±0.47	1.79±0.52	0.833
Duration of ART, years	1.34±1.54	1.69±1.96	1.32±1.51	0.083
Use of TDF, n (%)	171 (22.2%)	23 (41.1)	148 (20.7)	<0.001
Use of Lopinavir, n (%)	97 (12.6%)	13 (23.2)	43 (6.0)	0.013
Use of cotrimoxazole, n (%)	171 (22.2%)	18 (32.1)	153 (21.4)	0.062
AIDS defining disease, n (%)	69 (8.9%)	10 (17.9)	59 (8.3)	0.015

Data are mean±SD or n (%).

CKD; chronic kidney disease, ART; antiretroviral therapy, TDF; tenofovir

doi: 10.1371/journal.pone.0079885.t002

Factors associated with CKD

Univariate analysis identified older age per year-increase, female sex, body weight per 1 kg-decrement, use of TDF, use of lopinavir boosted with ritonavir, diabetes mellitus, and AIDS-defining diseases as factors significantly associated with CKD. After adjustment by multivariate analysis, older age per year-increase (OR=1.229; 95%CI, 1.170-1.291; p<0.001), body weight per 1 kg-decrement (OR=1.286; 95%CI, 1.193-1.386; p<0.001), and use of TDF (OR=2.715; 95%CI, 1.028-7.168; p=0.044) were associated significantly with CKD (Table **3**).

Discussion

We documented in the present study the prevalence of CKD and the associated risk factors in our Vietnamese cohort. CKD was identified in 7.3% of the patients between October 2011 and April 2012. Although severe renal dysfunction was observed in only 2 cases, we consider this finding quite alarming in our study setting, since it is more than double that reported in a previous study (3.1%) on the prevalence of CKD among Vietnamese healthy volunteers aged more than 40 years [19]. Our cohort comprised relatively younger and stable patients on ART with a mean age of 36.4 years.

Table 3. Factors associated with CKD based on uni- and multivariate analyses (n=771).

	Univariate analysis		Multivariate analysis		
Variables	**OR**	**95% CI**	**OR**	**95% CI**	**p value**
Age per year-increase	1.135	1.102 - 1.168	1.229	1.170 - 1.291	<0.001
Female	3.150	1.786 - 5.556	2.124	0.892 - 5.056	0.089
Body weight per 1 kg-decrement	1.170	1.119 - 1.223	1.286	1.193 - 1.386	<0.001
Use of TDF	2.670	1.522 - 4.685	2.715	1.028 - 7.168	0.044
Use of Lopinavir	2.257	1.165 - 4.370	1.439	0.460 - 4.497	0.531
Diabetes mellitus	3.180	1.251 - 8.084	1.614	0.353 - 7.383	0.537
AIDS defining disease	2.417	1.160 - 5.035	2.042	0.628 - 6.643	0.236
CD4+ cell count per cell/μl	1.000	0.998 - 1.001			
HIV-RNA level per log 10 copies/ml	1.055	0.641 - 1.736			
Duration of ART per year	1.138	0.982 - 1.318			
Use of cotrimoxazole	1.740	0.966 - 3.134			

OR = Odds ratio; CI = confidence interval; CKD; chronic kidney disease, ART; antiretroviral therapy, TDF; tenofovir

doi: 10.1371/journal.pone.0079885.t003

In addition to the high prevalence of CKD, a striking finding in this study was that TDF use has increased steeply since the 2010 WHO ART guidelines that recommended the use of TDF; TDF use was also an independent risk for CKD in Vietnamese, in addition to low body weight. We reported previously that Japanese patients with small body weight (<59 kg) treated with TDF were at high risk of renal dysfunction [16], whereas those with body weight of >67 kg had negligible risk, similar to the patients reported by Cooper et al [13]. One experimental study of rhesus macaques also reported that TDF-associated nephrotoxicity was dose-dependent [20]. The mean body weight of the patients enrolled in the present study was 55 kg, which is about 30 kg less than that of American males of similar age (88 kg) (URL:http://www.cdc.gov/nchs/data/nhsr/nhsr010.pdf). To prevent TDF-related CKD in patients with a small body weight, the efficacy and safety of low-dose TDF adjusted to low body weight should be evaluated in a clinical trial.

One study argued that the initial decline in eGFR following the commencement of TDF therapy stabilized later after the first 6 months [21]. However, whether or not the initial decline stabilizes later in patients with low body weight remains to be documented in a longitudinal study of our cohort. It is true that the future risk of TDF-related CKD is still uncertain. In this study, almost all patients who experienced renal dysfunction continued the same ART regimen because renal dysfunction was relatively moderate as shown in Table **1**. Although one severe case showed improvement of renal function after cessation of TDF, normalization of renal function after

withdrawal of TDF was reported to be incomplete in some cases [22]. Previous studies recommended dose reduction of drugs that are cleared by the kidney, such as lamivudine and TDF, when Ccl falls below 50 ml/min [23], to avoid further worsening of renal dysfunction. Early detection of eGFR decline is important for switching from TDF to AZT or abacavir to preserve renal function. Despite those concerns, however, there is no doubt that TDF is still an important drug with enough anti-HIV potency and less mitochondrial toxicity among NRTIs. In this regard, serum creatinine should be monitored even in resource-limited situations.

Furthermore, another study that compared patients with or without TDF use depicted that TDF was more likely to be used in the salvage regimen so far; patients on TDF had the longer duration of ART and more positive viral load (Table **2**). Based on this analysis, patients on TDF were more likely to develop CKD, although the mean body weight was not significantly different between the two groups. In addition, in terms of another antiretroviral agent, protease inhibitor (PI), also known as a risk factor for CKD [11], 97 (12.6%) patients used PIs (all PIs were ritonavir boosted lopinavir). Of 97 patients, 83 (85.6%) were co-administered with TDF. Although univariate analysis suggested that the use of PIs was associated significantly with CKD, multivariate analysis did not (Table **3**). The reason of this result could be explained by the short duration of co-administration and its effect as a confounding factor for TDF use.

The present study has several limitations. Due to its cross-sectional nature, we can only draw association of events and not demonstrate causative relationship between TDF and renal dysfunction. Further longitudinal studies are required to determine the impact of the aforementioned factors on renal function. Second, co-infection with HCV, a known risk factor for CKD, was not included in this analysis due to lack of available data in our cohort. The prevalence of HCV in Vietnamese is relatively high because injecting drug use is one of the main routes of infection in Vietnam. We are adding data for a longitudinal study on TDF toxicity in our cohort. Lastly, the Modification of Diet in Renal Disease formula (MDRD) or Chronic Kidney Disease Epidemiology Collaboration (CKD-epi) is commonly used for evaluation of renal function at present [24-26], however, the racial coefficient for Vietnamese is currently not available. In addition, serum creatinine was measured by the Jaffe method in our study, which is difficult to apply to MDRD or CKD-EPI since those formulations are based on measurement of serum creatinine by the more widely used enzyme method. For this reason, our study utilized Ccl to assess renal function.

Despite these limitations, the results of the present study call for attention to active pharmacovigilance of TDF. The results identified TDF exposure as a significant and independent risk for CKD in Vietnam, although the duration of TDF use is still relatively short. Further longitudinal study is required to evaluate the impact of TDF on renal function in Vietnam and other countries with small-body weight patients.

Supporting Information

Table S1. Median and inter-quartile range of serum creatinine of 771 patients at October 2011 and April 2012.
(DOCX)

Table S2. Baseline (October 2011) demographics and laboratory data of 771 patients with or without TDF use in whom serum creatinine was measured at October 2011 and April 2012.
(DOC)

Acknowledgements

The authors thank Ms. Keiko Saito and Ms. Nguyen Thi Huyen for the excellent assistance. The authors also thank all the clinical staff at the National Hospital of Tropical Diseases for their help in the completion of this study.

Author Contributions

Conceived and designed the experiments: DM JT TN HG SO. Performed the experiments: NL ND NK YK. Analyzed the data: DM TN FK. Contributed reagents/materials/analysis tools: YK HG. Wrote the manuscript: DM TN HG SO.

References

1. Wyatt CM, Winston JA, Malvestutto CD, Fishbein DA, Barash I et al. (2007) Chronic kidney disease in HIV infection: an urban epidemic. AIDS 21: 2101-2103. doi:10.1097/QAD.0b013e3282ef1bb4. PubMed: 17885301.
2. Mocroft A, Kirk O, Gatell J, Reiss P, Gargalianos P et al. (2007) Chronic renal failure among HIV-1-infected patients. AIDS 21: 1119-1127. doi:10.1097/QAD.0b013e3280f774ee. PubMed: 17502722.
3. Palella FJ Jr., Baker RK, Moorman AC, Chmiel JS, Wood KC et al. (2006) Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr 43: 27-34. doi:10.1097/01.qai.0000233310.90484.16. PubMed: 16878047.
4. Michaels SH, Clark R, Kissinger P (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 339: 405-406. doi:10.1056/NEJM199808063390612. PubMed: 9696654.
5. Gardner LI, Klein RS, Szczech LA, Phelps RM, Tashima K et al. (2003) Rates and risk factors for condition-specific hospitalizations in HIV-infected and uninfected women. J Acquir Immune Defic Syndr 34: 320-330. doi:10.1097/00126334-200311010-00011. PubMed: 14600579.
6. Déti EK, Thiébaut R, Bonnet F, Lawson-Ayayi S, Dupon M et al. (2010) Prevalence and factors associated with renal impairment in HIV-infected patients, ANRS C03 Aquitaine Cohort, France. HIV Med 11: 308-317. doi:10.1111/j.1468-1293.2009.00780.x. PubMed: 20002500.
7. Overton ET, Nurutdinova D, Freeman J, Seyfried W, Mondy KE (2009) Factors associated with renal dysfunction within an urban HIV-infected cohort in the era of highly active antiretroviral therapy. HIV Med 10: 343-350. doi:10.1111/j.1468-1293.2009.00693.x. PubMed: 19490182.
8. Lucas GM, Lau B, Atta MG, Fine DM, Keruly J et al. (2008) Chronic kidney disease incidence, and progression to end-stage renal disease, in HIV-infected individuals: a tale of two races. J Infect Dis 197: 1548-1557. doi:10.1086/587994. PubMed: 18422458.
9. Menezes AM, Torelly J Jr., Real L, Bay M, Poeta J et al. (2011) Prevalence and risk factors associated to chronic kidney disease in HIV-infected patients on HAART and undetectable viral load in Brazil. PLOS ONE 6: e26042. doi:10.1371/journal.pone.0026042. PubMed: 22022501.

10. Crum-Cianflone N, Ganesan A, Teneza-Mora N, Riddle M, Medina S et al. (2010) Prevalence and factors associated with renal dysfunction among HIV-infected patients. AIDS Patient Care STDs 24: 353-360. doi:10.1089/apc.2009.0326. PubMed: 20515419.
11. Mocroft A, Kirk O, Reiss P, De Wit S, Sedlacek D et al. (2010) Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. AIDS 24: 1667-1678. doi:10.1097/QAD.0b013e328339fe53. PubMed: 20523203.
12. Barrios A, García-Benayas T, González-Lahoz J, Soriano V (2004) Tenofovir-related nephrotoxicity in HIV-infected patients. AIDS 18: 960-963. doi:10.1097/00002030-200404090-00019. PubMed: 15060449.
13. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S et al. (2010) Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis 51: 496-505. doi:10.1086/655681. PubMed: 20673002.
14. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B et al. (2007) The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. AIDS 21: 1273-1281. doi: 10.1097/QAD.0b013e3280b07b33. PubMed: 17545703.
15. Chaisiri K, Bowonwatanuwong C, Kasettratat N, Kiertiburanakul S (2010) Incidence and risk factors for tenofovir-associated renal function decline among Thai HIV-infected patients with low-body weight. Curr HIV Res 8: 504-509. doi:10.2174/157016210793499259. PubMed: 21073439.
16. Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K et al. (2011) Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients. PLOS ONE 6: e22661. doi:10.1371/journal.pone.0022661. PubMed: 21799928.
17. Nishijima T, Gatanaga H, Komatsu H, Tsukada K, Shimbo T et al. (2012) Renal function declines more in tenofovir- than abacavir-based antiretroviral therapy in low-body weight treatment-naive patients with HIV infection. PLOS ONE 7: e29977. doi:10.1371/journal.pone.0029977. PubMed: 22242194.
18. National Kidney Foundation (2002) K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 39: S1-266. doi:10.1053/ajkd.2002.30571. PubMed: 11904577.
19. Ito J, Dung DT, Vuong MT, Tuyen do G, Vinh le D et al. (2008) Impact and perspective on chronic kidney disease in an Asian developing country: a large-scale survey in North Vietnam. Nephron Clin Pract 109: c25-c32. doi:10.1159/000134379. PubMed: 18497502.
20. Van Rompay KK, Durand-Gasselin L, Brignolo LL, Ray AS, Abel K et al. (2008) Chronic administration of tenofovir to rhesus macaques from infancy through adulthood and pregnancy: summary of pharmacokinetics and biological and virological effects. Antimicrob Agents Chemother 52: 3144-3160. doi:10.1128/AAC.00350-08. PubMed: 18573931.
21. Gallant JE, Moore RD (2009) Renal function with use of a tenofovir-containing initial antiretroviral regimen. AIDS 23: 1971-1975. doi: 10.1097/QAD.0b013e32832c96e9. PubMed: 19696652.
22. Wever K, van Agtmael MA, Carr A (2010) Incomplete reversibility of tenofovir-related renal toxicity in HIV-infected men. J Acquir Immune Defic Syndr 55: 78-81. doi:10.1097/QAI.0b013e3181d05579. PubMed: 20173649.
23. Dybul M, Fauci AS, Bartlett JG, Kaplan JE, Pau AK (2002) Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. Recommendations Panel Clinical Practices Treat HIV MMWR Recomm Rep 51: 1-55. PubMed: 12617573.
24. Stevens LA, Levey AS (2005) Measurement of kidney function. Med Clin North Am 89: 457-473. doi:10.1016/j.mcna.2004.11.009. PubMed: 15755462.
25. Stöhr W, Walker AS, Munderi P, Tugume S, Gilks CF et al. (2008) Estimating glomerular filtration rate in HIV-infected adults in Africa: comparison of Cockcroft-Gault and Modification of Diet in Renal Disease formulae. Antivir Ther 13: 761-770. PubMed: 18839777.
26. Soares AA, Eyff TF, Campani RB, Ritter L, Weinert LS et al. (2010) Performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study equations in healthy South Brazilians. Am J Kidney Dis 55: 1162-1163. doi:10.1053/j.ajkd.2010.03.008. PubMed: 20497836.

Ritonavir-Boosted Darunavir Is Rarely Associated with Nephrolithiasis Compared with Ritonavir-Boosted Atazanavir in HIV-Infected Patients

Takeshi Nishijima[1,3], Yohei Hamada[1], Koji Watanabe[1,3], Hirokazu Komatsu[2], Ei Kinai[1], Kunihisa Tsukada[1], Katsuji Teruya[1], Hiroyuki Gatanaga[1,3]*, Yoshimi Kikuchi[1], Shinichi Oka[1,3]

1 AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, **2** Department of Community Care, Saku Central Hospital, Nagano, Japan, **3** Center for AIDS Research, Kumamoto University, Kumamoto, Japan

Abstract

Background: Although ritonavir-boosted atazanavir (ATV/r) is known to be associated with nephrolithiasis, little is known about the incidence of nephrolithiasis in patients treated with ritonavir-boosted Darunavir (DRV/r), the other preferred protease inhibitor.

Methods: In a single-center cohort, the incidence of nephrolithiasis was compared between HIV-infected patients who commenced DRV/r-containing antiretroviral therapy and those on ATV/r. The effects of ATV/r use over DRV/r were estimated by univariate and multivariate Cox hazards models.

Results: Renal stones were diagnosed in only one patient (0.86 per 1000 person-years) of the DRV/r group (n=540) and 37 (20.2 per 1000 person-years) of the ATV/r group (n=517). The median [interquartile (IQR)] observation period in the DRV/r group was 27.1 months (IQR 18.1-38.4 months), and 40.6 months (IQR 17.5-42.7) for the ATV/r group. The total observation period was 1,163.6 person-years and 1,829.6 person-years for the DRV/r group and for the ATV/r group, respectively. In the 37 patients on ATV/r who developed nephrolithiasis, the median time from commencement of ATV/r to diagnosis was 28.1 months (IQR 18.4–42.7), whereas nephrolithiasis in the single patient of the DRV/r group occurred 11.2 month after the introduction of DRV/r. ATV/r use over DRV/r was significantly associated with nephrolithiasis by uni- and multivariate analyses (HR=26.01; 95% CI, 3.541–191.0; p=0.001) (adjusted HR=21.47; 95% CI, 2.879–160.2; p=0.003).

Conclusion: The incidence of nephrolithiasis was substantially lower in patients on DRV/r than those on ATV/r. The results suggest that DRV/r should be selected for treatment of HIV-infected patients at risk of chronic kidney disease.

Citation: Nishijima T, Hamada Y, Watanabe K, Komatsu H, Kinai E, et al. (2013) Ritonavir-Boosted Darunavir Is Rarely Associated with Nephrolithiasis Compared with Ritonavir-Boosted Atazanavir in HIV-Infected Patients. PLoS ONE 8(10): e77268. doi:10.1371/journal.pone.0077268

Editor: Mark Wainberg, McGill University AIDS Centre, Canada

Received August 12, 2013; **Accepted** September 9, 2013; **Published** October 10, 2013

Funding: This work was supported by a Grant-in-Aid for AIDS research from the Ministry of Health, Labor, and Welfare, Japan (H22-AIDS-001), and the Global Center of Excellence Program, the Ministry of Education, Science, Sports and Culture of Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: SO has received honoraria and research grants from MSD KK., Abbott Japan, Co., Janssen Pharmaceutical K.K., Pfizer, Co., and Roche Diagnostics K.K.; received honoraria from Astellas Pharmaceutical K.K., Bristol-Myers K.K., Daiichisankyo, Co., Dainippon Sumitomo Pharma, Co., GlaxoSmithKline, K.K., Taisho Toyama Pharmaceutical, Co., Torii Pharmaceutical, Co., and ViiV Healthcare. HG has received honoraria from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Torii Pharmaceutical, Co., and ViiV Healthcare, Co. All other authors declare no conflict of interest. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: higatana@acc.ncgm.go.jp

Introduction

Ritonavir-boosted darunavir (DRV/r) and ritonavir-boosted atazanavir (ATV/r) are the only two protease inhibitors (PI) selected as the preferred choices in the American Department of Health and Human Services (DHHS) guidelines for the initial treatment of patients infected with human immunodeficiency virus-1 (HIV-1) (http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). Both drugs are widely used in combination with other antiretroviral drugs, based on their high efficacy, tolerability, favorable lipid profile, and once-daily dosing [1-4]. However, nephrolithiasis has been reported in patients receiving ATV/r-containing antiretroviral therapy (ART) [5,6]. Several case reports documented high concentrations of ATV in renal stones, suggesting the involvement of ATV in nephrolithiasis [5-8]. We recently reported in a single center cohort study that the incidence of renal stones is approximately 10 times higher among patients on ATV/r-containing

antiretroviral therapy (ART) than those on other PIs-containing ART [9].

Our study on the effects of ART on renal stone formation included only a small number of patients on DRV/r-containing ART [9,10], and no data are available at present on the incidence of nephrolithiasis in patients treated with DRV/r. Of note, de Lastours et al [11] recently reported higher ATV and DRV levels in urine samples than in plasma, whereas plasma and urinary levels of lopinavir, another commonly used PI, were comparable. They also reported the presence of PI-containing crystals in the urine of a small proportion of patients on ATV and on DRV, but not on lopinavir/ritonavir (LPV/r). The data presented by de Lastours et al suggest that DRV can crystallize in urine leading to nephrolithiasis.

The aim of the present study was to determine the incidence of DRV/r- and ATV/r-related nephrolithiasis. Such comparison is important for two reasons: 1) These two PIs are most frequently prescribed PIs in resource-rich settings, and 2) nephrolithiasis is a risk factor for chronic kidney diseases (CKD) and end-stage renal disease (ESRD), which are important comorbidities associated with AIDS and death [12-16].

Methods

Ethics statement

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

Study Subjects

We performed a retrospective, single-center cohort study of HIV-1-infected patients using the medical records kept 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 HIV-infected patients, aged >17 years, who commenced treatment with DRV/r or ATV/r-containing ART between January 1, 2004 and June 30, 2012. Both treatment-naïve and treatment-experienced patients were included. The follow-up period started at the time of commencement of ART containing the abovementioned drugs for the first time during the study period, and patients were followed until June 30, 2013. Patients were excluded if they had; 1) commenced the abovementioned ART during the study period at other facilities, 2) been prescribed unboosted ATV, or 3) been under treatment for nephrolithiasis at the time of commencement of the abovementioned ART. ATV/r became available in Japan in January 2004, and DRV/r in December 2007.

The attending physician selected either ATV/r or DRV/r at baseline. The use of these drugs was based on the Japanese guidelines, which placed ATV/r and DRV/r as the preferred choice, at least for 5 years during the study period (http://www.haart-support.jp/pdf/guideline2013.pdf. in Japanese). The attending physician also selected the concurrent drugs including nucleoside reverse transcriptase inhibitors (NRTI), non-NRTI, integrase inhibitors, and CCR5 inhibitors. None of the patients received two PIs during the study period.

Measurements

The main investigator reviewed the medical records of all study patients to identify those with renal stones. Then two other investigators reviewed the set of medical records of each patient with renal stones to determine whether the case fitted into the following pre-defined criteria for nephrolithiasis: cases with a clinical diagnosis by the attending physician based on new onset of acute flank pain plus one of the following: 1) new-onset hematuria confirmed by urine dipstick test, 2) documented presence of stones or radiological findings suggestive of renal stones, such as hydronephrosis or obstruction or dilatation of the ureter, by either abdominal ultrasonography or computed tomography, 3) stone passage confirmed by either the patient or attending physician [9]. Patients with acute flank pain due to etiologies other than nephrolithiasis were excluded. At the time of diagnosis of nephrolithiasis, the attending physician selected either discontinuation or modification of ART. In our clinic, it is customary for the patient to visit the clinic once a month before the initiation of ART and until the suppression of HIV-1 viral load, but the visit interval is extended up to every three months after viral load suppression.

In this study, the primary exposure variable was ATV/r use over DRV/r. The potential risk factors for nephrolithiasis were determined according to previous studies and collected from the medical records, together with the basic demographics [7,8,17]. They included age, sex, body weight, body mass index (BMI)={bodyweight (kg) / [(height (m)]^2}, baseline laboratory data [CD4 cell count, HIV viral load, estimated glomerular filtration rate (eGFR), serum uric acid], and presence or absence of other medical conditions [concurrent use of tenofovir (TDF), past history of nephrolithiasis, previous exposure to indinavir (IDV), co-infection with hepatitis B defined by positive hepatitis B surface antigen, and co-infection with hepatitis C defined by positive hepatitis C viral load]. eGFR was calculated using the equation of the 4-variable Modification of Diet in Renal Diseases (MDRD) study [18]. For patients on ATV/r-containing ART, the value of serum total bilirubin was collected in two ways: for stone cases, total bilirubin value on the day was collected, and for non-stone cases, the value of total bilirubin 2 years after initiation of ATV/r was collected. For patients who discontinued ATV/r within 2 years, the value closest to the day of discontinuation was used. At our clinic, weight was 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 180 days, except for serum uric acid level, which were collected within 180 days from the day of starting ART.

Statistical analysis

Baseline characteristics were compared using the Student's *t*-test or χ^2 test (Fisher exact test) for continuous or categorical variables, respectively. The time to the diagnosis of nephrolithiasis was calculated from the date of commencement of DRV/r- or ATV/r-containing ART to the date of diagnosis of nephrolithiasis. Censored cases represented those who discontinued ATV/r or DRV/r, dropped out, were referred to other facilities, or at the end of follow-up period. The time from the start of ART to the diagnosis of nephrolithiasis was analyzed by the Kaplan Meier method for patients who started DRV/r (DRV/r group) and ATV/r (ATV/r group), 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 ATV/r use over DRV/r on the incidence of nephrolithiasis. The impact of each basic demographic parameter, 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 ATV/r use over DRV/r for nephrolithiasis, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for ATV/r use over DRV/r. Model 2 included age, sex, 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 after adjustment (these included tenofovir use, serum uric acid per 1 mg/dl, and past history of renal stones). Possible risk factors for ATV/r-related nephrolithiasis identified in previous studies were also added to model 3 (these included prior exposure to IDV) [7,8].

In addition, to examine the impact of serum total bilirubin on ATV/r-containing ART and the incidence of nephrolithiasis, the median serum total bilirubin values were compared between the renal stone and non-renal stone groups using the Mann-Whitney U test.

Statistical significance was defined as 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 nephrolithiasis. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 20.0 (SPSS, Chicago, IL).

Results

A total of 1,189 patients commenced either DRV/r- or ATV-containing ART between January 1, 2004 and June 30, 2012. Of the 1,057 patients who were included in the analysis, 540 (51%) started DRV/r-containing ART while 517 (48.9%) started ATV/r-containing ART (Figure 1). Table 1 shows the baseline characteristics of the study population. The ATV/r group included significantly younger (p=0.019), more patients of East Asian origin (p=0.009) with higher BMI (p=0.014), higher CD4 count (p=0.038), higher baseline serum uric acid (p=0.007), and a larger proportion of patients with past history of urinary stones (p=0.017) and previous exposure to IDV (p=0.036). In contrast, patients of the DRV/r group were significantly more likely to use tenofovir (p <0.001) and with higher viral load (p=0.002) (Table 1).

Thirty eight patients fulfilled the pre-defined criteria for nephrolithiasis. Nephrolithiasis was identified in 1 (0.2%) of the DRV/r group and 37 patients (7.1%) of the ATV/r group, with an estimated incidence of 0.86 and 20.2 per 1,000 person-years, respectively. The incidence of nephrolithiasis in the ATV/r group was approximately 20 times higher than that in the DRV/r group.

Of the patients with nephrolithiasis, 9 and 12 were diagnosed by hematuria and stone passage, respectively, as defined above. Furthermore, 17 were diagnosed by radiological studies, of which renal calcification was identified in 5 patients. Figure 2 shows the time from initiation or switching of DRV/r or ATV/r to the diagnosis of nephrolithiasis by the Kaplan Meier method. Patients of the ATV/r group were significantly more likely to develop renal stones, compared to those of the DRV/r group (p<0.001, Log-rank test).

The median time from the commencement of ART to the diagnosis of nephrolithiasis was 28.1 months [interquartile range (IQR), 18.4–42.7 months] for the ATV/r group and only one patient with nephrolithiasis in the DRV/r group was diagnosed 11.2 month after the introduction of DRV/r-containing ART. The total observation period was 1,163.6 patient-years [median, 27.1 months, IQR, 18.1–38.4 months] for the DRV/r group, and 1,829.6 patient-years [median, 40.6 months, IQR, 17.5–42.7 months] for the ATV/r group. Among the ATV/r group, the median total bilirubin value of the renal stone group was marginally higher than that of the non-renal stone group [2.7 (IQR 2-3.8) and 2.2 mg/dl (IQR 1.6-3.0), respectively, P=0.051].

Univariate analysis showed a significant relationship between ATV/r use and nephrolithiasis (HR=26.01; 95% CI, 3.541–191.0; p=0.001) (Table 2). Higher serum uric acid (HR=1.415; 95% CI, 1.173–1.705; p<0.001) and past history of nephrolithiasis (HR=2.658; 95% CI, 1.111–6.359; p=0.028) were also significantly associated with the nephrolithiasis. On the other hand, tenofovir use was negatively associated with nephrolithiasis (HR=0.435; 95% CI, 0.210–0.899; p=0.025) (Table 2). Multivariate analysis identified ATV/r use over DRV/r as an independent risk for nephrolithiasis after adjustment for age, male sex, and weight (adjusted HR=27.08 95% CI, 3.680–199.3; p=0.001) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR= 21.47; 95% CI, 2.879–160.2; p=0.003) (Table 3, Model 3).

The chemical composition of the renal stones of the single case on DRV/r was analyzed with high performance liquid chromatography with ultraviolet detection (HPLC-UV) method as described elsewhere [19,20], but the analysis did not identify DRV. Renal stones of patients on ATV/r were not analyzed.

Discussion

To our knowledge, this is the first study that investigated the incidence of DRV/r-associated nephrolithiasis. Only a single case of nephrolithiasis was detected among 540 patients on DRV/r-containing ART with total observation period of 1,163.6 patient-years. The incidence of nephrolithiasis in the DRV/r group was only 0.86 per 1,000 person-years, comparable to that in the general population in Japan (1.14 per 1,000 person-

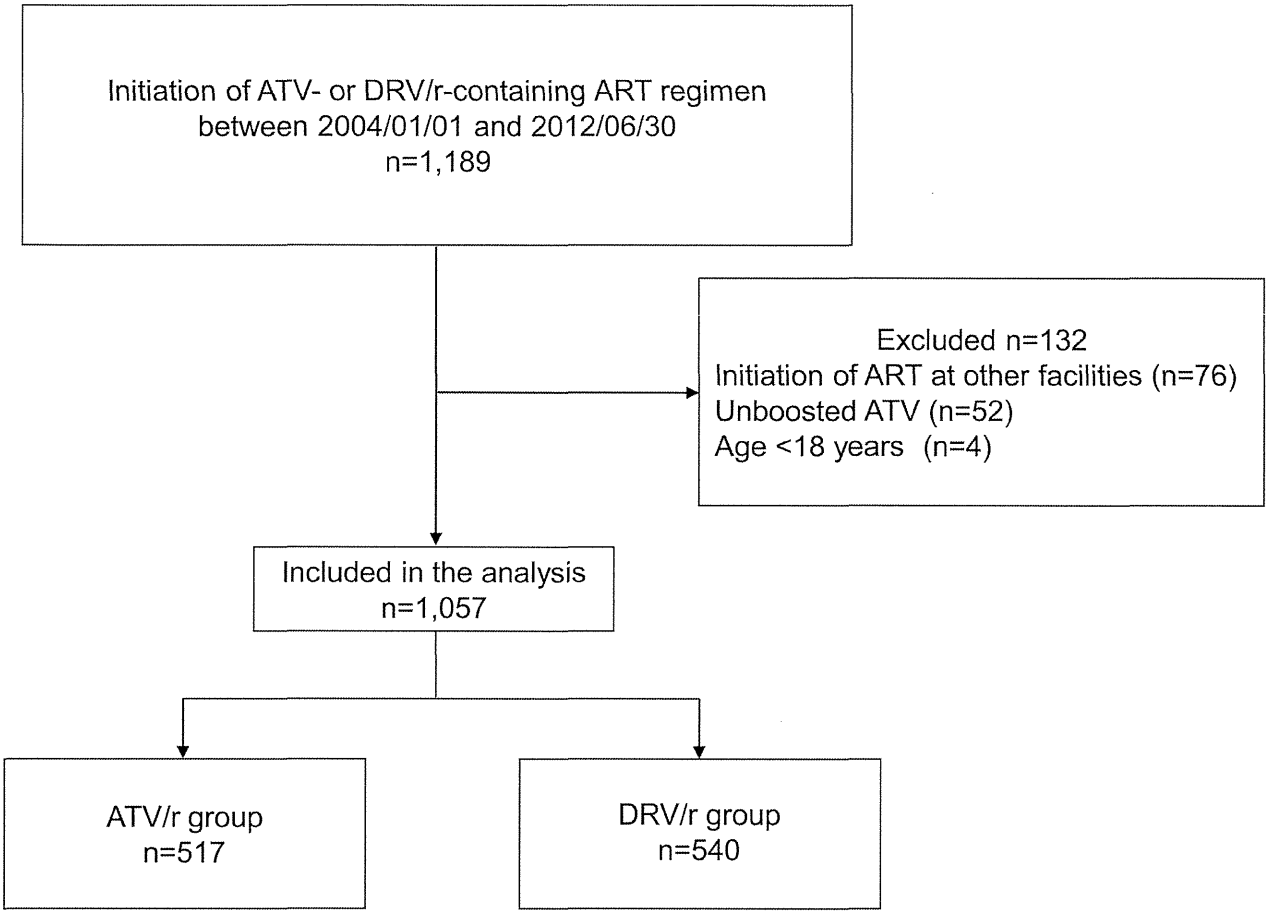

Figure 1. Flow diagram of patient selection. ART, antiretroviral therapy; ATV, atazanavir; DRV/r, ritonavir-boosted darunavir; ATV/r, ritonavir-boosted atazanavir.
doi: 10.1371/journal.pone.0077268.g001

years) [21], whereas that in the ATV/r group was 20.2 per 1,000 person-years, approximately 20 times higher. Univariate and multivariate analyses identified ATV/r use over DRV/r as an independent risk factor for nephrolithiasis with a high hazard ratio. Furthermore, in the single patient with nephrolithiasis on DRV/r, DRV was not detected as a component of renal stones.

This study showed that the risk of nephrolithiasis is substantially lower among patients on DRV/r- than those on ATV/r-containing ART based on clinically feasible criteria. This finding is important considering DRV/r and ATV/r are the two PIs considered the preferred regimen for the treatment-naïve patients (http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). Both PIs have similar characteristics; they are highly effective and tolerable with favorable lipid profile, and possess a high barrier to drug resistance [1-4]. One of the strengths of ATV/r is more abundant clinical evidence due to longer market availability than that of DRV/r. On the other hand, ATV/r often causes indirect hyperbilirubinemia, and requires acidic gastric environment for optimal absorption that requires some consideration on drug-drug interactions (http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf) (http://packageinserts.bms.com/pi/pi_reyataz.pdf). The substantially lower incidence of renal stones in patients on DRV/r than ATV/r adds another dimension to patient management in relation to the selection of a PI.

The development of renal stones, even a single episode, is a risk factor for CKD, doubling of serum creatinine level, and ESRD [12,13,16]. Many studies have also demonstrated that ATV/r use is a risk for renal dysfunction and CKD [22-25]. The high incidence of nephrolithiasis with ATV/r use identified in the present study may in part explain the risk of ATV/r for CKD. Thus, ATV/r should be introduced carefully in patients with concomitant predisposing factors for CKD. In this regard, there are no studies that show the association of DRV/r use with renal dysfunction or CKD, although this may in part be due to more recent introduction of DRV/r compared with ATV/r.

Why is nephrolithiasis less likely to occur with DRV/r compared to ATV/r? Although the mechanism of PI-induced nephrolithiasis is not fully understood, precipitation of pure PI is suggested as a possible etiology [8]. Up to 20% of IDV (an old PI well-known for its precipitation and renal stone formation) is

Table 1. Baseline demographics and laboratory data of patients who received ritonavir-boosted darunavir- or ritonavir-boosted atazanavir-containing antiretroviral therapy.

	DRV/r (n=540)	ATV/r (n=517)	P[a]
Age, years*	39 (33-46)	36 (31-44)	0.019
Male sex	498 (92.2)	480 (92.8)	0.727
Race (East Asian origin)	494 (91.5)	494 (95.6)	0.009
Body weight, kg*	62.1 (55.8-70)	64.0 (57.6-72)	0.074
body mass index, kg/m^2*	21.7 (19.8-24.1)	22.4 (20.4-24.6)	0.014
CD4 cell count, cells/μL*	251 (90-399)	260 (190-383)	0.038
HIV load, $\log_{10}$ copies/mL*	4.27 (1.70-5.17)	3.94 (1.70-4.66)	0.002
Treatment naïve	309 (57.2)	280 (54.2)	0.322
Tenofovir use	342 (63.3)	196 (37.9)	<0.001
eGFR, mL/min/1.73 m^2*	116 (102-131)	115 (103-130)	0.842
Serum uric acid, mg/dL*	5.7 (4.7-6.5)	5.9 (5.1-6.7)	0.007
HBV or HCV coinfection	78 (14.4)	64 (12.4)	0.367
Past history of nephrolithiasis	22 (4.1)	39 (7.5)	0.017
Previous exposure to IDV	25 (4.6)	41 (7.9)	0.030

Data are number (%) of patients or * median (interquartile range).

DRV/r, ritonavir-boosted darunavir; ATV/r, ritonavir-boosted atazanavir; eGFR, estimated glomerular filtration rate; HBV, hepatitis B virus, HCV, hepatitis C virus, HIV, human immunodeficiency virus; IDV, indinavir.

a. The χ^2 test or Fisher exact test was used for categorical data, and the Student *t* test was used for continuous variables.

doi: 10.1371/journal.pone.0077268.t001

excreted unchanged in the urine, a property that contributes to the high incidence of nephrolithiasis in patients treated with IDV [26] (http://www.merck.com/product/usa/pi_circulars/c/crixivan/crixivan_pi.pdf). Unchanged DRV and ATV are reported to be excreted in urine at similar proportions of 7.7% and 7% of the administered dose, respectively (http://packageinserts.bms.com/pi/pi_reyataz.pdf) (http://www.merck.com/product/usa/pi_circulars/c/crixivan/crixivan_pi.pdf). However, strong acidity (e.g., pH of 1.9) is required to achieve optimal dissolution of ATV, and its solubility in urine is known to decrease with increase in pH (http://packageinserts.bms.com/pi/pi_reyataz.pdf). Because urine is usually mildly acidic [9], the difference in the solubility of DRV and ATV in urine might explain the different incidence of nephrolithiasis in patients using these two PIs. Although de Lastours et al [11] described the presence of DRV crystals in the urine of 4 (7.8%) out of 51 patients on DRV/r and suggested that DRV/r use might be a risk for renal stones, the number of enrolled patients in their study was relatively small to allow firm conclusions.

The present study has several limitations. First, due to the retrospective nature of the study, the baseline characteristics of the enrolled patients were not controlled. It is possible that more patients with potential risks for nephrolithiasis were included in the ATV/r group. In the ATV/r group, more patients were hyperuricemic, had history of renal stones, and previous exposure to IDV, which are known risk factors for nephrolithiasis. However, multivariate analysis clearly showed that ATV/r use is an independent risk factor with high hazard ratio even after adjustment for variables including the above three. Second, the median observation period was longer in the ATV/r group than in the DRV/r group (40.6 versus 27.1 months), suggesting that the risk of nephrolithiasis in the ATV/r group could be overestimated. Further studies are warranted to elucidate whether much longer use of DRV/r induces nephrolithiasis. However, it is noteworthy that in patients with nephrolithiasis, the median time from the commencement of ATV/r or DRV/r to the diagnosis of nephrolithiasis was 28.1 months (IQR: 18.4-42.7 months), which was similar to that of the DRV/r group [median 27.1 (IQR: 18.1-38.4)], backing up the result of the present study: the risk of nephrolithiasis is substantially lower among patients on DRV/r than those on ATV/r. Third, stone composition analysis was conducted in only one patient with renal stones (treated with DRV/r), therefore, it is possible that renal stones caused by other etiologies are included.

In conclusion, the present study demonstrated that the risk of nephrolithiasis, an important risk factor of CKD, is approximately 20 times lower among patients on DRV/r- than those on ATV/r-containing ART, providing DRV/r one advantage over ATV/r in the selection of PI. ATV/r use was identified as a significant and independent risk factor for nephrolithiasis in a robust statistical model that included ATV/r use over DRV/r as a primary exposure. ATV/r should be prescribed with caution in patients with predisposing factors for nephrolithiasis and those with CKD.