Smoking is a Risk Factor for Development of Adult T-cell Leukemia/Lymphoma in Japanese Human T-cell Leukemia Virus Type-1 Carriers

Hisayoshi Kondo[1], Midori Soda[2], Norie Sawada[3], Manami Inoue[3,4], Yoshitaka Imaizumi[5], Yasushi Miyazaki[6], Masako Iwanaga[7], Yasuhito Tanaka[8], Masashi Mizokami[9], Shoichiro Tsugane[3]

Running title: Smoking is a risk factor for ATLL development

1 Biostatistics Section, Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan

2 Department of Epidemiology, Radiation Effects Research Foundation, Nagasaki, Japan

3 Epidemiology and Prevention Group, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan

4 Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

5 Department of Hematology, Nagasaki University Hospital, Nagasaki, japan

6 Department of Hematology, Atomic Bomb Disease and Hibakusha Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, japan

7Department of Frontier Life Science, Unit of Basic Sciences, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, japan

8 Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

9 The Research Center for Hepatitis and Immunology, National Center for Global Health and

Medicine, Ichikawa, Chiba, Japan

Corresponding author: Dr. Hisayoshi Kondo, Biostatistics Section, Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki University, Sakamoto 1-12-4, Nagasaki 852-8523, Japan

Tel: +81(0)95 819 7127

Fax: +81(0)95 819 7131

E-mail: hkondo@nagasaki-u.ac.jp

Reprint requests: Dr. Hisayoshi Kondo, Biostatistics Section, Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki University, Sakamoto 1-12-4, Nagasaki 852-8523, Japan

Abstract

Background and Purpose

Adult T-cell leukemia/lymphoma (ATLL) is an aggressive hematological malignancy caused by human T-cell leukemia virus type-1 (HTLV-1); no effective methods have yet been identified to prevent development of ATLL in carriers of HTLV-1. This study investigated the association between cigarette smoking and the risk of ATLL development among Japanese carriers of HTLV-1.

Methods

This study examined the association between smoking and development of ATLL in a cohort of 1,332 Japanese HTLV-1 carriers aged 40-69 years free of ATLL at baseline from two different HTLV-1-endemic areas of Japan. Cox proportional hazards models adjusted for sex, geographical area, age at baseline and alcohol drinking were used to estimate the effect of cigarette smoking on ATLL development

Results

Between 1993 and 2012, 25 new ATLL cases were identified among these subjects. The overall crude incidence rate for ATLL was 1.08 per 1,000 person-years among HTLV-1 carriers and was higher among male carriers than among female carriers (2.21 vs. 0.74). The risk of ATLL development increased significantly with increasing numbers of cigarettes smoked per day (hazard ratio for every increment of 20 cigarettes, 2.03; 95% confidence interval [CI]: 1.13-3.66 overall, 2.07 (95%CI:1.13-3.73) in male carriers).

Conclusions

Cigarette smoking may influence ATLL development among HTLV-1 carriers in Japan.

Keywords: ATLL; Cigarette Smoking; HTLV-1; Japanese

Introduction

Adult T-cell Leukemia/Lymphoma (ATLL) is a hematological malignancy associated with human T-cell leukemia virus type 1 (HTLV-1) [1]. HTLV-1 was discovered in the early 1980's; [2,3] it has been identified worldwide, and an estimated 5 to 10 million individuals are infected [4]. Southwest Japan [5], the Caribbean Islands, Central Africa, and South America are considered the primary endemic regions [4].

HTLV-1 can be transmitted from mother to child via breastfeeding, as well as by sexual intercourse or blood transfusions. In Japan, the country with the highest HTLV-1 endemicity worldwide, transmission via blood transfusion has been nearly eliminated through viral screening of donated blood since 1986 [6]. Mother-to-child transmission has also decreased in endemic areas after implementation of programs in 1987 to discourage HTLV-1 carrier mothers from breast-feeding [7]. Nevertheless, approximately one million people are infected with HTLV-1 [8], and approximately 1,000 new cases of ATLL and 1,000 deaths due to ATLL are reported annually in Japan [9]. Because ATLL is an aggressive entity with poor prognosis, effective therapeutic and preventive strategies are necessary.

Most HTLV-1-infected subjects remain lifelong asymptomatic carriers, but a proportion may progress to ATLL after a long latency period; the cumulative lifetime risk of ATLL was estimated to be 4.0% and 4.2% for Jamaican males and females, respectively, infected before the age of 20 years [10]. On the other hand, the cumulative incidence of ATLL among HTLV-1 carriers in Japan was estimated at 2.5% (3%-5% in males, 1%-2% in females) [11]. A variety of risk factors and determinants for progression to ATLL from HTLV-1 carrier status have been reported [12-14], including early life exposure to HTLV-1, male sex, advanced age, laboratory abnormalities such as a high levels of soluble interleukin-2 receptor, higher proviral load, and many genetic and epigenetic alterations [15-18]. However, no definitive factors have yet been

identified. Moreover, to our knowledge, no prospective cohort study has identified lifestyle factors associated with ATLL development among HTLV-1 carriers in the general population.

Cigarette smoking is a well-known risk factor for a variety of malignancies, including hematological malignancies [19]. However, no study has prospectively investigated the association between cigarette smoking and ATLL development among HTLV-1 carriers. The present study, therefore, investigated this relationship in asymptomatic HTLV-1 carriers in a long-term population-based cohort study in Japan.

Materials and Methods

Study population

The Japan Public Health Center-Based Prospective Study (JPHC study) [20] consists of two cohorts: residents aged 40-59 years from five public health center (PHC) regions who started the study in 1990 (Cohort I), and residents aged 40-69 years from an additional six PHC areas who started in 1993–1994 (Cohort II). The details of these cohorts have been described previously [20].

This study used Cohort II data. Cohort II (n = 78,825) included 63,216 participants (80% response rate) who completed a baseline questionnaire on medical history, smoking and drinking habits, etc. Of these, 38.6% (n = 24,374) voluntarily provided 10 mL blood samples at health checkups during the baseline survey. The blood samples were divided into plasma and buffy layers and preserved at -80°C until analysis. Study participants were informed of the objectives and methods of the study in writing, and those who answered the questionnaire and donated blood were regarded as having provided informed consent to participate. Of these, we selected only those who tested positive for HTLV-1 antibodies and had provided data on their basic characteristics (n = 9,705); moreover, we restricted the study population to only those

subjects who were HTLV-1-seropositive, leaving a total of 1,451 subjects. Of these HTLV-1-seropositive subjects, two had already been diagnosed with ATLL before the start of the follow-up period, and 117 had undocumented or incomplete information regarding their smoking and/or drinking habits. Thus, a total of 1,332 Japanese HTLV-1 carriers (344 men and 988 women) were included in the final analysis (Figure 1).

Assessment of Exposure

Questions on smoking habits included current and former smoking status, age at smoking initiation and quitting, and average number of cigarettes smoked per day. We defined current and former smokers as ever smokers. Smoking intensity for ever smokers was evaluated by the number of cigarettes smoked per day or by pack-years defined by multiplying the number of cigarettes per day divided by 20 by the number of years of smoking.

Questions on drinking habits included current and former drinking status, frequency, types of drinks, and average consumption per day. We defined drinkers with a drinking frequency of 3 or more days per week as regular drinkers. Drinking intensity for regular drinkers was evaluated based on weekly alcohol consumption defined by multiplying the daily amount of ethanol intake by the weekly frequency

The prevalence of regular drinkers among ever smokers were significantly higher than among never smokers in overall and female, but not in male (Table 1).

Cohort follow-up and identification of ATLL cases

Subjects were followed up from the baseline until December 31, 2012. Changes in residence status, including survival, were identified annually through residential registries. Because the follow-up rate was 99.9%, selection bias due to participants lost to follow-up was considered

negligible.

The JPHC cancer registry identified ATLL cases among the study subjects from two data sources: active patient notification from major local hospitals in the study areas and population-based cancer registries with permission from the local governments responsible for these registries. ATLL cases were coded based on the International Classification of Diseases for Oncology, Third Edition (ICD-O-3) morphology/behavior code (code: 9827/3) [21]. Death certificates were used as supplementary sources of information. The proportion of cases for which information was only available from death certificates was 8.0%, indicating satisfactory cancer registry system quality during the study period.

Laboratory assays for HTLV-1

A commercial passive particle agglutination assay common in Japan was used to screen for HTLV-1 antibodies (SERODIA HTLV-I, Fujirebio Inc., Tokyo, Japan).

Statistical methods

Person-years of follow-up were calculated for each person from the date of baseline until the date of ATLL diagnosis, death, moving out of the study area, or the end of follow-up, whichever occurred first. The baseline factors included in the analyses were age at baseline, sex, geographic area, smoking status or intensity, and drinking intensity. To evaluate the effect of smoking on ATLL development, we used two different Cox proportional hazards models and estimated the hazard ratios (HRs) with 95% confidence intervals (CIs) [22]. First, ever smokers (former and current smokers) were compared with never smokers. Second, smoking status was treated as "continuous intensity" and represented by the number of cigarettes per day or cigarette pack-years. The hazard ratios for ever smokers and for each level of smoking intensity

were estimated by univariate and multivariate analysis. Multivariate analyses were adjusted for sex, age at baseline and weekly alcohol intake after geographical stratification. The proportional hazards assumptions for each covariate were evaluated by a Kolmogorov-type supremum test and applied to all covariates. Furthermore, we performed analyses for males only to clarify the effects of smoking on ATLL development.

All statistical tests were two-sided and $P < 0.05$ were considered statistically significant. The calculations were performed using the PHREG procedure in the SAS software package (version 9.3; SAS Institute, Inc., Cary, NC, USA).

Approval

This study was approved by the Nagasaki University Graduate School of Biomedical Sciences Ethics Committee (Protocol No.14121245), and the use of JPHC study data was approved by the Ethics Committee of the National Cancer Center, Japan.

Results

A total of 25 cases of ATLL were identified during 22,961 person-years of follow-up from 1993 to 2012 (average follow-up period: 17.2 years) of 1,332 subjects seropositive for HTLV-1 at baseline.

The distributions of baseline characteristics among these HTLV-1 carriers are summarized in Table 1. The majority of female HTLV-1 carriers were never smokers, whereas never smokers comprised nearly one-quarter of male subjects. Male smokers who belonged to the category equivalent to more than 40 pack-years were slightly older than other male subjects. The proportions of male smokers (former and current) and heavy smokers (≧20 cigarettes per day or ≧40 pack-years) increased with age.

The overall crude incidence rate was 1.08 per 1,000 person-years (PYs) of follow-up. The crude incidence rate was significantly higher in male HTLV-1 carriers (2.21 per 1,000 PYs) than in female carriers (0.74 per 1,000 PYs) (Table 2).

Among the two categories of smoking history, ATLL incidence was significantly higher for ever smokers (HR 3.08, 95%CI: 1.38–6.88) than for never smokers in univariate analysis, but not in multivariate analysis (HR 1.64, 95%CI: 0.45–6.06) (Table 2).

With respect to the effects of smoking intensities, there were significant increasing risks of ATLL development by increments of each intensity in both univariate and multivariate analysis. Namely, the HRs for every increment of 20 cigarettes smoked per day which was the mean number among ever smokers were 2.37 (95%CI: 1.58-3.52) in univariate analysis and 2.03 (95%CI: 1.13-3.66) in multivariate analysis. Similarly, the HRs for every increment of 40 pack-years which was the mean value among ever smokers were 2.79 (95%CI: 1.89-4.28) in univariate analysis and 2.39 (95%CI: 1.32-4.44) in multivariate analysis (Table 2).

With the analysis restricted to males only, ATLL incidence was considerably higher for ever smokers than for never smokers; however, the correlation was not significant according to both univariate and multivariate analysis (HR 2.18, 95%CI: 0.48–9.96, HR 2.06, 95%CI: 0.45–9.47, respectively; Table 2.

With respect to the effects of smoking intensity, significant increasing risks of ATLL development according to increments for each intensity level were observed in both univariate and multivariate analysis. The HRs for every increment of 20 cigarettes smoked per day were 2.07 (95%CI: 1.15-3.80) in univariate analysis and 2.07 (95%CI: 1.13-3.73) in multivariate analysis. Similarly, the HRs for every increment of 40 pack-years were 2.48 (95%CI: 1.43-4.45) in univariate analysis and 2.48 (95%CI: 1.32-4.62) in multivariate analysis (Table 2).

With respect to the effects of drinking intensities, there were no significant increasing risks of

ATLL development by increments of weekly alcohol intake in each multivariate analysis. The HRs for every increment of 300g per weekly intake which was the mean amount among regular drinkers were 1.00 (95%CI: 0.55-2.46) in overall, 1.00 (95%CI: 0.55-2.46)) in male and 1.00 (95%CI: 0.30-35.8)) in female groups (data not shown).

Discussion

To our knowledge, the present study is the first to prospectively examine the effects of cigarette smoking on ATLL development in a sub-cohort of HTLV-1 carriers within a long-term, large-scale Japanese prospective cohort. We found a significant positive association between smoking intensity and ATLL development among overall and male HTLV-1 carriers based on the number of cigarettes smoked per day and pack-years, but did not observe a significant difference between never smokers and ever smokers. Smoking intensities in male ever smokers were distributed over a wide range, i.e., the number of cigarettes per day ranged from 4 to 40 and pack-years from 1 to 195; therefore, we believed that the ever smokers did not all possess the same characteristics. In addition, with regard to the effect of cigarette smoking on the human body, we presume that those are probabilistic rather than deterministic; therefore, smoking intensity better represents the effects of smoking than smoking status (history).

Until now, the association between development of ATLL and cigarette smoking has been controversial. Tokudome et al. reported that smoking prevalence was significantly higher in ATLL cases than that in the general population and that there was a clear dose-response relationship between the amount (pack-years) of smoking and risk of ATLL development [23]. However, Hisada et al. reported that smoking prevalence did not differ significantly between ATLL cases and HTLV-1 carriers in their case-control study [24]. Our main finding of significantly increased hazard ratios for development of ATLL with smoking intensity (amount

of cigarettes), supports those of Tokudome et al. [23] and suggests that smoking is a risk factor for ATLL development among HTLV-1 carriers.

Studies of cigarette smoking and risk of other lymphoid malignancies have yielded inconsistent results. Previously, Morton et al. reported increased risk of non-Hodgkin lymphoma (NHL) with smoking in an international pooled analysis [25], whereas the association was not observed in a large-scale European study [26]. Recent studies on the etiologic heterogenecity of lymphoma subtype have investigated subtype-specific risks of lymphoma associated with smoking. For example, several studies reported that smoking was associated with increased risks of follicular lymphoma, but not for other NHL subtypes [25,27]. Another pooled case-control study investigated the association between smoking and risk of Hodgkin lymphoma (HL) by subtype, and identified a positive association between mixed cellularity and Epstein-Barr virus-positive HL [28].

Other recent studies have reported that smoking is associated with increased risk of a higher incidence of T-cell lymphoma, but not of other NHL subtypes [29,30]. Although they did not assess ATLL, their results may in part support our findings, since ATLL is a type of T-cell lymphoma. Unfortunately, the biological mechanism for the association between smoking and risk of T-cell malignancy remains unclear.

Although HTLV-1 infection alone is insufficient for ATLL development, specific genetic ATLL abnormalities that are additionally acquired (and required) have not been fully elucidated [34-36]. Moreover, it remains unknown whether these genetic abnormalities arise due to cigarette smoking or to HTLV-1 infection itself, and if they independently affect ATLL development. Whatever the reasons, our findings on the association between cigarette smoking and ATLL development suggest that quitting or decreasing smoking intensity, which is easily practicable in HTLV-1-endemic countries, may prevent ATLL development among the more

than 10 million HTLV-1 carriers worldwide.

Our study had several strengths. First, the prospective design enabled us to evaluate the effects of cigarette smoking on the incidence rate of ATLL among HTLV-1 carriers for the first time, contrary to the majority of previous Japanese studies using cross-sectional designs [37]. The incidence rate of ATLL in the present cohort study, 1.08 (2.2 for men and 0.74 for women) per 1,000 person-years of HTLV-1 carriers, was consistent with a previous report [37]. In addition, our study design enabled us to minimize potential biases common in case-control studies, such as recall and selection biases. Second, the JPHC study employed detailed follow-up procedures and had virtually complete ascertainment of incident ATLL cases because of the high precision of the cancer registry system in this study. The proportion of death certification only (DCO) was only 8%.

Some limitations of our study should be discussed. First, the present study had a small sample size, which limited our power to examine the effects of smoking on ATLL development, especially in female HTLV-1 carries, whose smoking status was skewed towards never-smokers. Additional large-scale, prospective studies are necessary to confirm our results. Second, we did not evaluate ATLL development according to known risk markers, such as soluble interleukin 2 receptors (IL-2R) levels and proviral load [15,16]. Therefore, studies specific to ATLL in HTLV-1 carriers that include these risk markers are needed to determine whether smoking is a significant risk factor in addition to existing risk markers. Third, smoking habits were assessed by means of self-administered questionnaires, which could have introduced exposure misclassification and resulted in underestimation of risk. However, a recent study indicated that self-reported smoking habits were substantially reliable [38]. Finally, smoking and drinking habits were evaluated only at baseline in a survey that lasted an average of 17 years. Therefore, the possibility of confounding by change in these habits cannot be ruled out.

In conclusion, the results of this study indicate that cigarette smoking may influence ATLL development among HTLV-1 carriers in Japan.

Acknowledgements

The authors thank the staff of each JPHC study area and the central office for their painstaking efforts to conduct the baseline survey and follow-up. We are indebted to the Ibaraki, Niigata, Osaka, Kochi, Nagasaki, and Okinawa Cancer Registries for providing their incidence data. This study was supported by the National Cancer Center Research and Development Fund (23-A-31[toku] and 26-A-2) (since 2011) and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan (from 1989 to 2010).

Compliance with ethical standard

Conflict of interest

The authors of this paper have no conflict of interest.

References

1. Takatsuki K, Uchiyama T, Sagawa K, Yodoi J. Adult T cell leukemia in Japan. In Seno S, Takaku S and Irino S eds. *Topics in Hematology, Amsterdam*: Excepta Medica, 1977; 73-77.
2. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci* USA. 1980; **77**: 7415–9.
3. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell

lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci USA*.1982; **79**: 2031–35.

4. Gessain A, Cassar O. Epidemiological aspects and world distribution on HTLV-1 infection. *Front Microbiol* 2012; **3**: 1-23.
5. Tajima K.The 4th nation-wide study of adult T-cell leukemia/lymphoma (ATL) in Japan: estimates of risk of ATL and its geographical and clinical features. The T-cell and B-cell Malignancy Study Group. *Int J Cancer* 1990; **36**: 643-9.
6. Inaba S, Sato H, Okochi K, Fukada K, et al. Prevention of transmission of human T-lymphotropic virus type 1 (HTLV-1) through transfusion, by donor screening with antibody to the virus: One-year experience. *Transfusion* 1989; **29**: 7–11.
7. Hino S. Establishment of the milk-borne transmission as a key factor for the peculiar endemicity of human T-lymphotropic virus type 1 (HTLV-1): the ATL Prevention Program Nagasaki. *Proc Jpn Acad Ser B Phys Biol Sci.* 2011; **87(4)**: 152-66.
8. Satake M, Yamaguchi K, Tadokoro K. Current prevalence of HTLV-1 in Japan as determined by screening of blood donors. *J Med Virol*. 2012; **84(2)**: 327-35.
9. Yamada Y, Atogami S, Hasegawa H, Kamihira S, Soda M, Satake M, Yamaguchi K. Nationwide survey of adult T-cell leukemia/lymphoma (ATL) in Japan. *Rinsho Ketsueki.* 2011 Nov; **52(11)**: 1765-71. [Japanese]
10. Murphy EL, Hanchard B, Figueroa JP, et al. Modeling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type 1. Int J Cancer 1989:43(2); 250-53.
11. Yamaguchi K and Watanabe T. Human T lymphotropic virus type-1 and adult T-cell leukemia in Japan. Int J Hematol 2002: 76(2): 240-45.
12. Wilks R. Patterns of HTLV-1 infection among family members of patients with adult T-cell leukemia/lymphoma and HTLV-1 associated myelopathy/tropical spastic

paraparesis. Int J Cancer 1996: 65(2); 272-73

13. Iwanaga M, Watanabe T, Yamaguchi K. Adult T-cell leukemia: a review of epidemiological evidence. *Front Microbiol*. 2012 Sep 10; **3**: 322.
14. Yamagishi M, Watanabe T. Molecular hallmarks of adult T cell leukemia. *Front Microbiol.* 2012 Sep 17; **3**: 334.
15. Arisawa K, Katamine S, Kamihira S, et al. A nested case-control study of risk factors for adult T-cell leukemia/lymphoma among human T-cell lymphotropic virus type-I carriers in Japan. *Cancer Causes Control* 2002; **13(7)**: 657-663.
16. Iwanaga M, Watanabe T, Utsunomiya A, et al. Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan. *Blood*. 2010 Aug 26; **116(8)**: 1211-9.
17. Seiki M, Hattori S, Hitayama Y, Yoshida M. Human adult T-cell leukemia virus: complete nucleotides sequence of the provirus genome integrated in leukemia cell DNA. *Proc Natl Acad Sci USA* 1983: **80**; 3618-22.
18. Wong-Staal F, Gallo RC. Human T-lymphotropic retroviruses. *Nature* 1985: **317**: 395-403
19. Fircanis S, Merriam P, Khan N, Castillo JJ. The relation between cigarette smoking and risk of acute myeloid leukemia: an updated meta-analysis of epidemiological studies. *Am J Hematol.* 2014 Aug; 89(8): E125-32.
20. Tsugane S, Sawada N. The JPHC study: design and some findings on the typical Japanese diet. Jpn Clin Oncol 2014 Sep; **44(9)**: 777-82.
21. Fritz AG, International Classification of Diseases for Oncology: ICD-O (3rd ed.)World Health Organization, Geneva, Switzerland (2000).
22. Cox DR. Regression models and life tables (with discussion). *J R Stat Soc Series B stat Methodol* 1972: **34**; 187-220.

23. Tokudome S, Shimamoto Y, Sumida I. Smoking and T-cell leukemia/lymphoma. *Eur J Cancer Prev* 1993; **2**: 84-86.

24. Hisada M, Okayama A, Shioiri S, Spiegelman DL, Stuver SO, Mueller NE. Risk factors for adult T-cell leukemia among carriers of human T-lymphotropic virus type I. *Blood* 1998; **92**: 3357-61

25. Morton LM, Hartge P, Holford TR, et al. Cigarette smoking and risk of non-Hodgkin lymphoma: a pooled analysis from the International Lymphoma Epidemiology Consortium (interlymph). *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 925–33.

26. Besson H, Brennan P, Becker N, Nieters A, De Sanjosé S, Font R, Maynadié M, Foretova L, Cocco PL, Staines A, Vornanen M, Boffetta P. Tobacco smoking, alcohol drinking and non-Hodgkin's lymphoma: A European multicenter case-control study (Epilymph). *Int J Cancer.* 2006 Aug 15; **119(4)**: 901-8.

27. Diver WR, Patel AV, Thun MJ, Tears LR, Gapstur SM. The association between cigarette smoking and non-Hodgkin lymphoid neoplasms in a large US cohort study. *Cancer Causes Control.* 2012; 23: 1231-40.

28. Kamper-Jørgensen M, Rostgaard K, Glaser SL, Zahm SH, Cozen W, et al. Cigarette smoking and risk of Hodgkin lymphoma and its subtypes: a pooled analysis from the International Lymphoma Epidemiology Consortium (InterLymph). *Ann Oncol.* 2013 Sep; **24(9)**: 2245-55.

29. Castillo JJ, Dalia S. Cigarette smoking is associated with a small increase in the incidence of non-Hodgkin lymphoma: a meta-analysis of 24 observational studies. *Leuk Lymphoma.* 2012 Oct; **53(10)**: 1911-9.

30. Diver WR, Patel AV, Thun MJ, Teras LR, Gapstur SM. The association between cigarette smoking and non-Hodgkin lymphoid neoplasms in a large US cohort study. *Cancer*

Causes Control. 2012 Aug; **23(8)**: 1231-40.

31. Sopori M. Effects of cigarette smoke on the immune system. *Nat Rev Immunol*. 2002 May; **2(5**): 372-7.
32. Stämpfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. *Nat Rev Immunol*. 2009 May; **9(5)**: 377-84.
33. Nordman JC, Muldoon P, Clark S, Damaj MI, Kabbani N. The α4 nicotinic receptor promotes CD4+ T-cell proliferation and a helper T-cell immune response. *Mol Pharmacol.* 2014 Jan; **85(1)**: 50-61.
34. Ceasrman E, Chadburn A, Inghirami G, et al. Structual and functional analysis of oncogenes and tumor suppressor genes in adult T-cell leukemia/lymphoma shows freaquent p53 mutations. *Blood* 1992: **80**; 3205-16.
35. Hatta Y, Koeffler HP. Role of tumor suppressor genes in the development of adult T cell leukemia/lymphoma (ATL). *Leukemia* 2002; **16**: 1069-85.
36. Okamoto T. Multi-step carcinogenesis model for adult T-cell leukemia. *Jpn J Cancer Res* 1989; **80**: 191-95.
37. Tajima K, Kuroishi T. Estimation of rate of incidence of ATL among ATLV(HTLV-1) carriers in Kyushu, *Jpn. Jpn J Clin Oncol* 1985; **15**: 423-30.
38. Yeager DS, Krosnick JA. The validity of self-reported nicotine product use in the 2001-2008 National Health and Nutrition Examination Survey. *Med Care* 2010; **48**: 1128-32.

Appendix

Members of the Japan Public Health Center-based Prospective Study (JPHC Study, principal investigator: S. Tsugane) Group are: S. Tsugane, N. Sawada, M. Iwasaki, S. Sasazuki, T. Yamaji, T. Shimazu and T. Hanaoka, National Cancer Center, Tokyo; J. Ogata, S. Baba, T. Mannami, A.

Okayama, and Y. Kokubo, National Cerebral and Cardiovascular Center, Osaka; K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, I. Hashimoto, T. Ikuta, Y. Tanaba, H. Sato, Y. Roppongi, T. Takashima and H. Suzuki, Iwate Prefectural Ninohe Public Health Center, Iwate; Y. Miyajima, N. Suzuki, S. Nagasawa, Y. Furusugi, N. Nagai, Y. Ito, S. Komatsu and T. Minamizono, Akita Prefectural Yokote Public Health Center, Akita; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe, Y. Miyagawa, Y. Kobayashi, M. Machida, K. Kobayashi and M. Tsukada, Nagano Prefectural Saku Public Health Center, Nagano; Y. Kishimoto, E. Takara, T. Fukuyama, M. Kinjo, M. Irei, and H. Sakiyama, Okinawa Prefectural Chubu Public Health Center, Okinawa; K. Imoto, H. Yazawa, T. Seo, A. Seiko, F. Ito, F. Shoji and R. Saito, Katsushika Public Health Center, Tokyo; A. Murata, K. Minato, K. Motegi, T. Fujieda and S. Yamato, Ibaraki Prefectural Mito Public Health Center, Ibaraki; K. Matsui, T. Abe, M. Katagiri, M. Suzuki, K. and Matsui, Niigata Prefectural Kashiwazaki and Nagaoka Public Health Center, Niigata; M. Doi, A. Terao, Y. Ishikawa, and T. Tagami, Kochi Prefectural Chuo-higashi Public Health Center, Kochi; H. Sueta, H. Doi, M. Urata, N. Okamoto, F. Ide, H. Goto and R Fujita, Nagasaki Prefectural Kamigoto Public Health Center, Nagasaki; H. Sakiyama, N. Onga, H. Takaesu, M. Uehara, T. Nakasone and M. Yamakawa, Okinawa Prefectural Miyako Public Health Center, Okinawa; F. Horii, I. Asano, H. Yamaguchi, K. Aoki, S. Maruyama, M. Ichii, and M. Takano, Osaka Prefectural Suita Public Health Center, Osaka; Y. Tsubono, Tohoku University, Miyagi; K. Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita; Y. Honda, K. Yamagishi, S. Sakurai and N. Tsuchiya, University of Tsukuba, Ibaraki; M. Kabuto, National Institute for Environmental Studies, Ibaraki; M. Yamaguchi, Y. Matsumura, S. Sasaki, and S. Watanabe, National Institute of Health and Nutrition, Tokyo; M. Akabane, Tokyo University of Agriculture, Tokyo; T. Kadowaki and M. Inoue, The University of Tokyo, Tokyo; M. Noda and T. Mizoue, National Center for Global

Health and Medicine, Tokyo; Y. Kawaguchi, Tokyo Medical and Dental University, Tokyo; Y. Takashima and Y. Yoshida, Kyorin University, Tokyo; K. Nakamura and R. Takachi, Niigata University, Niigata; J. Ishihara, Sagami Women's University, Kanagawa; S. Matsushima and S. Natsukawa, Saku General Hospital, Nagano; H. Shimizu, Sakihae Institute, Gifu; H. Sugimura, Hamamatsu University School of Medicine, Shizuoka; S. Tominaga, Aichi Cancer Center, Aichi; N. Hamajima, Nagoya University, Aichi; H. Iso and T. Sobue, Osaka University, Osaka; M. Iida, W. Ajiki, and A. Ioka, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka; S. Sato, Chiba Prefectural Institute of Public Health, Chiba; E. Maruyama, Kobe University, Hyogo; M. Konishi, K. Okada, and I. Saito, Ehime University, Ehime; N. Yasuda, Kochi University, Kochi; S. Kono, Kyushu University, Fukuoka; S. Akiba, Kagoshima University, Kagoshima; T. Isobe, Keio University; Y. Sato, Tokyo Gakugei University, Tokyo.

Figure Captions

Figure 1. Flow chart of analytical subject enrollment

Figure 1

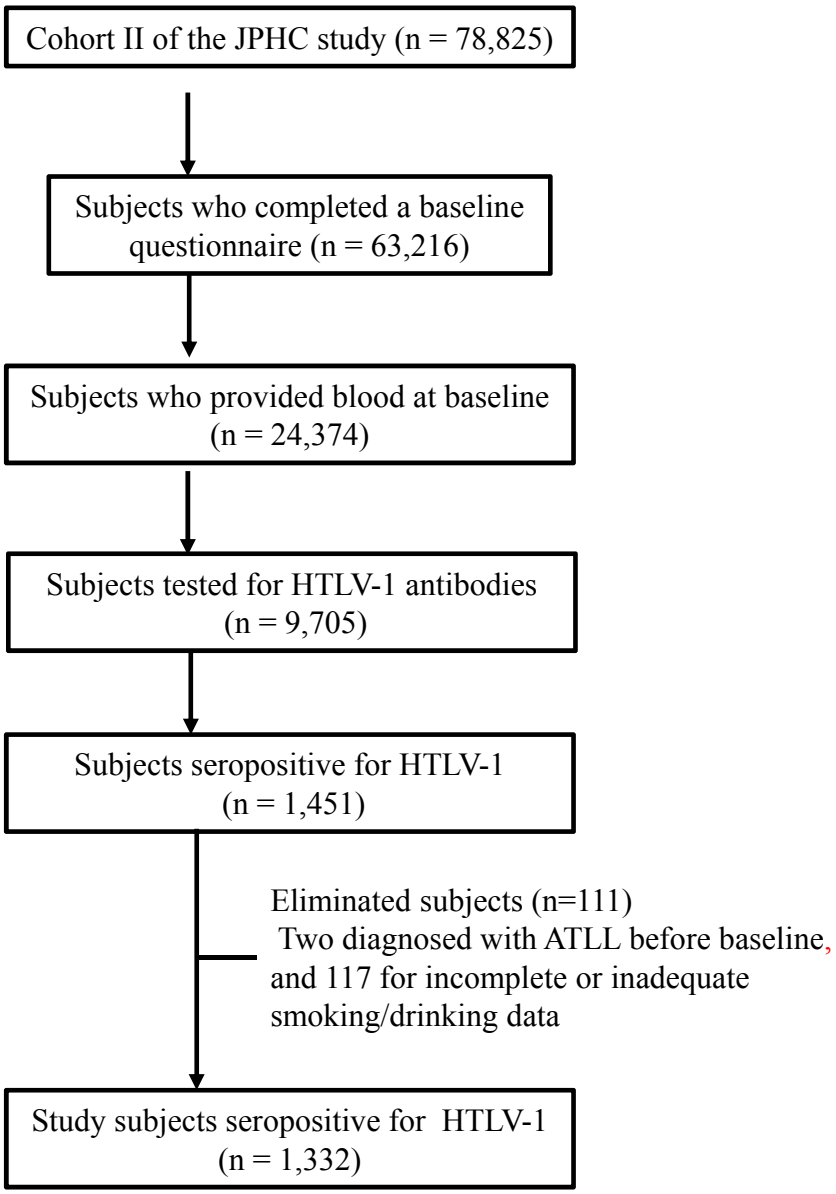
Cohort II of the JPHC study (n = 78,825)
Subjects who completed a baseline questionnaire (n = 63,216)
Subjects who provided blood at baseline (n = 24,374)
Subjects tested for HTLV-1 antibodies (n = 9,705)
Subjects seropositive for HTLV-1 (n = 1,451)
Eliminated subjects (n=111)
Two diagnosed with ATLL before baseline, and 117 for incomplete or inadequate smoking/drinking data
Study subjects seropositive for HTLV-1 (n = 1,332)

Table 1. Baseline characteristics according to cigarette smoking and alcohol drinking status of 1,332 HTLV-1 carriers

Characteristic	Number of subjects	Status of cigarette smoking				Status of alcohol drinking		
		Status		Intensity in ever smokers		Status		Aicohol consumption
		Never smoking	Ever smoking	Mean number of cigarettes per day	Mean pack-years[a]	Never drinking	Regular drinking[b]	Mean weekly intake[c]
Overall	1,332	1,060 (80%)	272 (20%)	22.5 (12.5[d])	37.4 (24.6[d])	1,133 (85%)	199 (15%)	331 (237[d])
Age at baseline								
Mean years	59.5 (7.4[d])	59.3 (7.3)	60.6 (7.7)	–	–	59.7(7.3)	58.9 (7.9)	–
Age rank (years)								
40-49	168	137 (82%)	31 (18%)	21.5 (10.8[d])	21.1 (14.3[d])	134 (80%)	34 (20%)	293 (185[d])
50-59	421	353 (84%)	68 (16%)	23.1 (13.1)	31.8 (20.9)	369 (88%)	52 (12%)	369 (274)
60-69	743	570 (77%)	173 (23%)	22.4 (12.5)	42.5 (25.7)	630 (85%)	113 (15%)	324 (232)
Ever smokers (%)	–	–	–	–	–	12.2	67.3	–
Regular drinkers (%)	–	6.1	49.3	–	–	–	–	–
Male	344	95 (28%)	249 (72%)	23.6 (12.2[d])	39.9 (24.0[d])	172 (50%)	172 (50%)	352 (240[d])
Age at baseline								
Mean years	60.5 (7.5)	59.1 (7.7)	61.1 (7.4)	–	–	61.4 (7.1)	59.6 (7.7)	–
Age rank (years)								
40-49	41	17 (41%)	24 (59%)	23.7 (11.0[d])	24.2 (14.3[d])	16 (39%)	25 (61%)	329 (194[d])
50-59	83	24 (29%)	59 (71%)	25.1 (12.2)	35.4 (19.9)	44 (53%)	39 (47%)	415 (277)
60-69	220	54 (25%)	166 (75%)	23.0 (12.4)	43.8 (25.4)	112 (51%)	108 (49%)	334 (233)
Ever smokers (%)	–	–	–	–	–	69.8	75	–
Regular drinkers (%)	–	45.3	51.8	–	–	–	–	–
Female	988	965 (98%)	23 (2%)	10.7 (8.9[d])	10.2 (8.6[d])	961 (97%)	27 (3%)	199 (169[d])
Age at baseline								
Mean years	59.2 (7.4)	59.3 (7.3)	55.8 (9.1)	–	–	59.4(7.3)	54.1 (7.5)	–
Age rank (years)								
40-49	127	120 (94%)	7 (6%)	13.9 (5.8[d])	10.4 (8.3[d])	118 (93%)	9 (7%)	195 (115[d])
50-59	338	329 (97%)	9 (3%)	10.1 (12.5)	8.0 (7.7)	325 (96%)	13 (4%)	230 (220)
60-69	523	516 (99%)	7 (1%)	8.4 (5.7)	12.8 (10.5)	518 (99%)	5 (1%)	127 (65)
Ever smokers (%)	–	–	–	–	–	1.9	18.5	–
Regular drinkers (%)	–	2.3	21.7	–	–	–	–	–

a: number of cigarettes per day divided by 20 and multiplied by the number of years of smoking
b: 3 days or more/week drinking
c: ammount of daily alchol intake and multiplied by the weekly frequency
d: standard deviation

Table 2. Effects of cigarette smoking on development of ATLL in 1,332 HTLV-1 carriers

	Person years of follow-up	Number with ATLL	Crude incidence rate[a]	Univariate hazard ratio (95%CI[b])	Multivariate hazard ratio (95%CI)
Overall	23,111	25	1.08		
Sex					
Male	5,454	12	2.20	3.03 (1.38－6.66)	1.34 (0.42－4.29)[c]
Female	17,657	13	0.74	1	1
Smoking history					
Never smokers	18,971	15	0.79	1	1
Ever smokers	4,140	10	2.42	3.08 (1.38－6.88)	1.64 (0.45－6.06)[d]
Smoking intensity					
Increments of 20 cigarettes per day[f]				2.37 (1.58－3.52)	2.03 (1.13–3.66)[d]
Increments of 40 pack-years[f]				2.79 (1.89－4.28)	2.39 (1.32–4.44)[d]
Male					
Smoking history					
Never smokers	1,688	2	1.18	1	1
Ever smokers	3,766	10	2.66	2.18 (0.48－9.96)	2.06 (0.45－9.47)[e]
Smoking intensity					
Increments of 20 cigarettes per day				2.07 (1.15－3.80)	2.07 (1.13–3.73)[e]
Increments of 40 pack-years				2.48 (1.43－4.45)	2.48 (1.32–4.62)[e]
Female					
Smoking history					
Never smokers	17,283	13	0.75	-	-
Ever smokers	374	0	0	-	-

ATLL: adult T-cell leukemia/lymphoma, HTLV-1: human T-cell leukemia virus type-1
a: per 1,000 person-years, b: confidence interval
c: adjusted for age at baseline, cigarettes per day, amount of weekly alcohol intake and geographic area
d: adjusted for age at baseline, sex, amount of weekly alcohol intake and geographic area
e: adjusted for age at baseline, amount of weekly alcohol intake and geographic area
f: mean value of ever smokers