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

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Definitive risk factors for the development of adult T-cell leukemia (ATL) among asymptomatic human T-cell leukemia virus type I (HTLV-1) carriers remain unclear. Recently, HTLV-1 proviral loads have been evaluated as important predictors of ATL, but a few small prospective studies have been conducted. We prospectively evaluated 1218 asymptomatic HTLV-1 carriers (426 males and 792 females) who were enrolled during 2002 to 2008. The proviral load at enrollment was significantly higher in males than females (median, 2.10 vs 1.39 copies/100 peripheral blood mononuclear cells [PBMCs]; $P < .001$), in those 40 to 49 and 50 to 59 years of age than that of those 40 years of age and younger ($P = .02$ and .007, respectively), and in those with a family history of ATL than those without the history (median, 2.32 vs 1.33 copies/100 PBMCs; $P = .005$). During follow-up, 14 participants progressed to overt ATL. Their baseline proviral load was high (range, 4.17-28.58 copies/100 PBMCs). None developed ATL among those with a baseline proviral load lower than approximately 4 copies. Multivariate Cox analyses indicated that not only a higher proviral load, advanced age, family history of ATL, and first opportunity for HTLV-1 testing during treatment for other diseases were independent risk factors for progression of ATL. (*Blood.* 2010;116(8): 1211-1219)

Introduction

Human T-cell leukemia virus type I (HTLV-1), the first human retrovirus to be identified, is etiologically associated with adult T-cell leukemia (ATL), HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP), and HTLV-1 uveitis/HTLV-1–associated uveitis (HU/HAU).[1-3] Worldwide, endemic areas for the virus are unevenly distributed, which include southwest Japan, the Caribbean islands, South America, and a part of Central Africa.[4] In Japan, the number of HTLV-1 carriers was estimated to be approximately 1.2 million people during the late 1980s.[5] The majority of HTLV-1 carriers remain asymptomatic throughout their lives. The lifetime risks of developing ATL and HAM/TSP are estimated to be approximately 2.5% to 5%[6,7] and 0.3% to 2%,[8,9] respectively.

Several molecular biologic studies have reported that various cellular dysfunctions induced by viral genes (eg, *tax* and *HBZ*), genetic and epigenetic alterations, and the host immune system may be involved in the leukemogenesis of ATL.[10-12] Clinical and epidemiologic studies have also reported a variety of possible risk factors for ATL, including vertical transmission of HTLV-1 infection, male gender, a long latent period, increased leukocyte counts or abnormal lymphocyte counts, and higher levels of anti–HTLV-1 antibody titers and soluble interleukin-2 receptor-α.[13-19] However, there are no clear determinants that separate those who develop ATL from those who remain healthy carriers.

Recently, HTLV-1 proviral load levels have been evaluated as important predictors of development of ATL and HAM/TSP. Some cross-sectional studies showed that HTLV-1 proviral load levels were higher in ATL and HAM/TSP compared with asymptomatic HTLV-1 carriers.[20,21] However, the proviral load levels of asymptomatic HTLV-1 carriers exhibited a very wide range,[20,22,23] and these levels may vary by sex, race, habitats, and comorbidities.[24] The proviral load levels of asymptomatic HTLV-1 carriers were also examined serially in some prospective studies; however, the

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number of reported cases was very small.[25-28] Although these previous studies suggest a possible important role for HTLV-1 proviral load in the development of ATL and HAM/TSP, the association between HTLV-1 proviral load and diseases development remains unclear.

The identification of risk factors for developing ATL among virus carriers is necessary to prevent these diseases in HTLV-1 endemic areas. To investigate detailed viral- and host-specific determinants of disease development, larger and longer prospective studies are warranted. In 2002, we established a nationwide cohort study for asymptomatic HTLV-1 carriers in Japan named the Joint Study on Predisposing Factors of ATL Development (JSPFAD).[29] The main objective of this project is to establish reliable predisposing factors for developing ATL by prospectively following a large number of asymptomatic HTLV-1 carriers. Here, for the first-time, we report the study method, baseline demographic characteristics, and distribution characteristics of baseline HTLV-1 proviral load of asymptomatic HTLV-1 carriers. We have also evaluated progression to ATL and its risk predictors.

Methods

Participants and study design

The JSPFAD is a nationwide prospective study of HTLV-1 carriers, which was approved by the Ministry of Education, Culture, Sports, Science and Technology of Japan. The project was established in August 2002 by Japanese clinicians and basic researchers of 41 institutions composed of 14 university hospitals and 27 educational hospitals located in various areas of Japan (supplemental Appendix, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Objectives of the project are to establish reliable predisposing factors for development of ATL by prospectively following a large number of asymptomatic HTLV-1 carriers. This includes performing clinical examinations and biomarker assays, as well as establishing a biomaterial resource bank of plasma, viable peripheral blood mononuclear cells (PBMCs), frozen PBMCs pellets, and genomic DNA from PBMCs of HTLV-1–infected persons for the future evaluations with new molecular biology techniques.

Hematologists at the collaborating institutions were responsible for enrolling participants after receiving approval from their Institutional Review Boards. The study protocol was approved by the Ministry of Education, Culture, Sports, Science and Technology of Japan. Eligible participants were those who had known of their HTLV-1 infection and had confirmed the HTLV-1–positive serology at any of the medical institutions. Potential participants visited any of the collaborating institutions directly or via the website of the JSPFAD (www.htlv1.org/). They received adequate explanations for the enrollment procedure from the hematologists at the collaborating institutions. Enrollment was conditional on participants giving written informed consent in accordance with the Declaration of Helsinki. The primary participants were asymptomatic HTLV-1 carriers. A small number of patients with definite ATL, HAM/TSP, and HU/HAU were also enrolled as controls.

Data collection and sample storage

After providing written informed consent, participants were expected to fill out a questionnaire regarding demographic information, to provide peripheral blood samples, and to periodically visit the institution for follow-up. After reconfirming the asymptomatic HTLV-1 carrier status of the participants, hematologists at the collaborating institutions assigned a unique identification number to each participant and subsequently sent all materials (individual questionnaire sheets, clinical data, and blood samples drawn into ethylenediaminetetraacetic acid and heparin tubes) to the JSPFAD office (Department of Medical Genome Sciences, Laboratory of Tumor Cell Biology, Graduate School of Frontier Sciences, University of Tokyo, Japan).

The self-administered questionnaire included items on demographic characteristics, birthplaces of the participants and their mothers, family history regarding HTLV-1 status and HTLV-1–associated diseases, length of marriage, partner's HTLV-1 status, first opportunity for HTLV-1 testing, and histories of disease manifestations other than HTLV-1–associated diseases. Additional questionnaire items, information on prior blood transfusion, and smoking habits (present, past, or nonsmoking) were also included after April 2008.

Clinical data included information on the date of visit, complete blood cell count, differential cell counts (including abnormal lymphocytes per 100 leukocytes), lactate dehydrogenase, HTLV-1 serologic test, comorbidities other than HTLV-1–associated diseases, and the development of any HTLV-1–associated diseases during follow-up. Blood samples were collected at enrollment, annually thereafter (in principal), and as needed. Blood samples sent to the study office at the University of Tokyo were separated into plasma, PBMCs, and genomic DNA and then used for viral marker assays at the University of Tokyo or stored for the biomaterial bank at the Japanese Red Cross Fukuoka Blood Center.

Viral marker assays

HTLV-1 proviral load of PBMC samples was measured by real-time polymerase chain reaction (PCR) using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems Japan), as previously described with minor modifications.[30,31] Genomic DNA from PBMCs was isolated using a QIAGEN Blood Kit (QIAGEN). Quantitative real-time PCR was performed using multiplex PCR with 2 sets of primers specific for the HTLV-1 provirus and the human gene encoding the RNase P enzyme. The primers and the probe for the gene encoding RNase P were purchased from Applied Biosystems; those for the pX region of the HTLV-1 provirus were described previously.[30,31] Genomic DNA of normal control PBMCs mixed with a plasmid DNA, which contained almost the whole genome of the HTLV-1 provirus (*SacI* site of 5′-LTR to *SacI* site of 3′-LTR), was used as control template. The copy number of the plasmid DNA was calculated based on the size and weight of the plasmid DNA, as measured by spectrophotometry. The proviral loads were expressed as copy numbers per 100 PBMCs, based on the assumption that infected cells harbored 1 copy of the integrated HTLV-1 provirus per cell. Samples with a higher proviral load (> 20 copies/100 PBMCs) were subjected to Southern blot analysis to examine the clonality of the infected cells. Assays to detect the integrated band of HTLV-1 provirus genome were described previously.[32] Genomic DNA samples (10 mg) were digested with *PstI* or *Eco*RI restriction enzymes and were size-fractionated on 0.7% agarose gels. They were then transferred onto a nylon membrane by the Southern blot technique. Hybridization to randomly primed ^{32}P-labeled DNA probes for the whole proviral genome (*SacI* to *SacI* fragment of the HTLV-1 proviral genome) was performed, followed by appropriate stringency washing steps and autoradiography. Soluble interleukin-2 receptor was measured by a commercial laboratory (SRL Inc) using an enzyme-linked immunosorbent assay (Endogen) and reported as units per milliliter.

Statistical analysis

Analyses were performed for participants who enrolled as of December 2008. Age at enrollment was categorized into 5 groups: younger than 40, 40 to 49, 50 to 59, 60 to 69, and 70 years or older. Geographic location was divided into 4 areas: northern (Hokkaido and Tohoku), metropolitan (Tokyo, Osaka, and Nagoya), southern (Kyushu and Okinawa), and others (supplemental Figure 1). First opportunity for HTLV-1 testing was divided into 3 categories: by screening for HTLV-1 (regional-mass, multiphasic, blood donor, and maternal screenings), by the presence of HTLV-1–infected family members (including spouse), and by the patient status under treatment for diseases unrelated to HTLV-1. A positive family history was considered to be present when participants had information on first-degree relatives (parents, siblings, or offspring) who were HTLV-1 carriers or had HTLV-1–associated diseases (ie, ATL, HAM/TSP, and HU/HAU). Any leukemia and/or lymphoma other than ATL were also taken into consideration. A positive comorbidity at enrollment was considered to be present when any information on diseases other than HTLV-1–associated diseases

was available at enrollment. HTLV-1 proviral loads (copy numbers/100 PBMCs) were used as a continuous variable (raw and the power-transformed data) or by categorizing them into quartiles. We applied a square-root transformation to the raw data of proviral loads to reduce the skewness. Continuous data were presented as median (range) values and compared using a Mann-Whitney test. Categorical data were compared using a χ^2 test or Fisher exact test. We calculated person-years of follow-up for each participant from the date of enrollment to the date of ATL diagnosis, the date of last follow-up, or September 30, 2009, whichever came first. Cumulative progression to ATL was estimated using Kaplan-Meier curves. To estimate the effect of baseline HTLV-1 proviral load and selected demographic factors on ATL development, we performed Cox proportional hazards analyses, and expressed as hazard ratios (HR) and 95% confidence intervals (CI), which were calculated by robust sandwich variance estimates. To check for possible incompleteness in the multivariate model, we also performed analyses using sub-datasets. All statistical analyses were performed using SAS Version 9.1 (SAS Institute Japan) with a 2-tailed significance level of .05.

Results

Baseline demographic characteristics

From August 2002 to December 2008, 1259 participants of asymptomatic HTLV-1 carriers were enrolled in this study. However, HTLV-1 proviral load was not measured for 41 participants. Thus, a total of 1218 participants (426 males and 792 females) were included in this analysis. Demographic characteristics of the participants at enrollment are shown in Table 1. The median ages at enrollment in the cohort were 59.6 years (range, 6.9-92.8 years) for males or 58.3 years (range, 17.8-90.3 years) for females. The largest percentage of study participants was from the southern area, which is a well-known HTLV-1 endemic area in Japan, followed by the metropolitan area. The southern area also had the largest percentage for birthplaces for most participants and their mothers.

One-half of the participants came to know of their HTLV-1 infections through screening for HTLV-1, and one-fourth was informed of their infections while receiving treatments for diseases other than HTLV-1–associated diseases. More than half of the participants did not know their family status of HTLV-1 infection. Only 119 female participants knew about the HTLV-1 infection status of their husbands, of whom 53 (45%) of the husbands were positive for HTLV-1 (data not shown). However, we were not able to obtain reliable information on male-to-female transmission for the female participants. We obtained information on comorbidities at enrollment from 257 participants, of which 45 had comorbid infectious diseases (eg, strongyloidiasis, chronic bronchitis, hepatitis C virus infection, lymphadenitis), 29 had autoimmune diseases (rheumatoid arthritis, chronic thyroiditis, Sjögren syndrome, and other autoimmune or chronic inflammatory diseases), 80 had a variety of definite malignant diseases other than ATL (non-Hodgkin lymphoma, acute myeloid leukemia, gastric cancer, lung cancer, or other malignancies), 16 had skin diseases, and 87 had other common diseases (eg, hypertension, diabetes).

Distributions of baseline HTLV-1 proviral load

Figure 1 shows distribution of baseline HTLV-1 proviral load in 1218 participants. There was a wide range of skewness in the raw data, with a median of 1.60 copies/100 PBMCs (range, 0-55.8 copies/100 PBMCs; 25th-75th percentile, 0.29-4.54 copies/100 PBMCs; Figure 1A). The square-root transformation reduced the skew in the raw data, with a median of 1.26 copies/100

Table 1. Baseline demographic characteristics of asymptomatic HTLV-1 carriers

Variable	Male, no. (%)	Female, no. (%)
Total	426	792
Age, y		
Younger than 40	48 (11.3)	119 (15.0)
40-49	70 (16.4)	130 (16.4)
50-59	99 (23.2)	174 (22.0)
60-69	88 (20.7)	172 (21.7)
70 or older	121 (28.4)	197 (24.9)
Place of enrollment		
Northern area	10 (2.3)	32 (4.0)
Metropolitan area	75 (17.6)	144 (18.1)
Southern area	333 (78.2)	597 (75.4)
Other areas	8 (1.9)	19 (2.4)
Birthplace of participants		
Northern area	18 (4.2)	33 (4.2)
Metropolitan area	30 (7.0)	80 (10.1)
Southern area	240 (56.3)	400 (50.5)
Other areas	20 (4.7)	54 (6.8)
Unknown	118 (27.7)	225 (28.4)
Birthplace of participants' mothers		
Northern area	16 (3.8)	32 (4.0)
Metropolitan area	13 (3.1)	39 (4.9)
Southern area	247 (58.0)	426 (53.8)
Other areas	28 (6.6)	64 (8.1)
Unknown	122 (28.6)	231 (29.2)
First opportunity for HTLV-1 testing		
Screening for HTLV-1	209 (49.1)	452 (57.1)
Regional mass screening	77	164
Multiphasic screening	24	44
Blood donor screening	108	128
Maternal screening	0	116
Revelation of HTLV-1–positive family	33 (7.7)	101 (12.7)
During treatment of other diseases	117 (27.5)	148 (18.7)
Unknown	67 (15.7)	91 (11.5)
Family history of HTLV-1–associated diseases*		
Absent	98 (23.0)	154 (19.5)
Absent for a first-degree relative but having an infected spouse	6 (1.4)	23 (2.9)
Carrier only	27 (6.3)	74 (9.3)
HU/HAU only	2 (0.5)	1 (0.1)
HAM	2 (0.5)	7 (0.9)
ATL	34 (8.0)	74 (9.3)
Leukemia or lymphoma	9 (2.1)	26 (3.3)
Unknown family history	248 (58.2)	433 (54.7)
Comorbidity†		
Absent	331 (77.7)	630 (79.5)
Present	95 (22.3)	162 (20.5)
Infectious diseases	20	25
Autoimmune diseases	3	26
Malignant diseases	36	44
Skin diseases	8	8
Other disease	28	59

HTLV-1 indicates human T-cell leukemia virus type 1; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; and ATL, adult T-cell leukemia.

*Family history was restricted to a first-degree relative. "Present" indicates that participants have a parent, sibling, or offspring diagnosed with HTLV-1-associated diseases. Family members with HAM and HU/HAU were included into the category of "HAM." Family members with ATL and HAM and/or HU/HAU were included into the category of "ATL."

†Comorbidity indicates that participants have any diseases other than HTLV-1–associated diseases at enrollment.

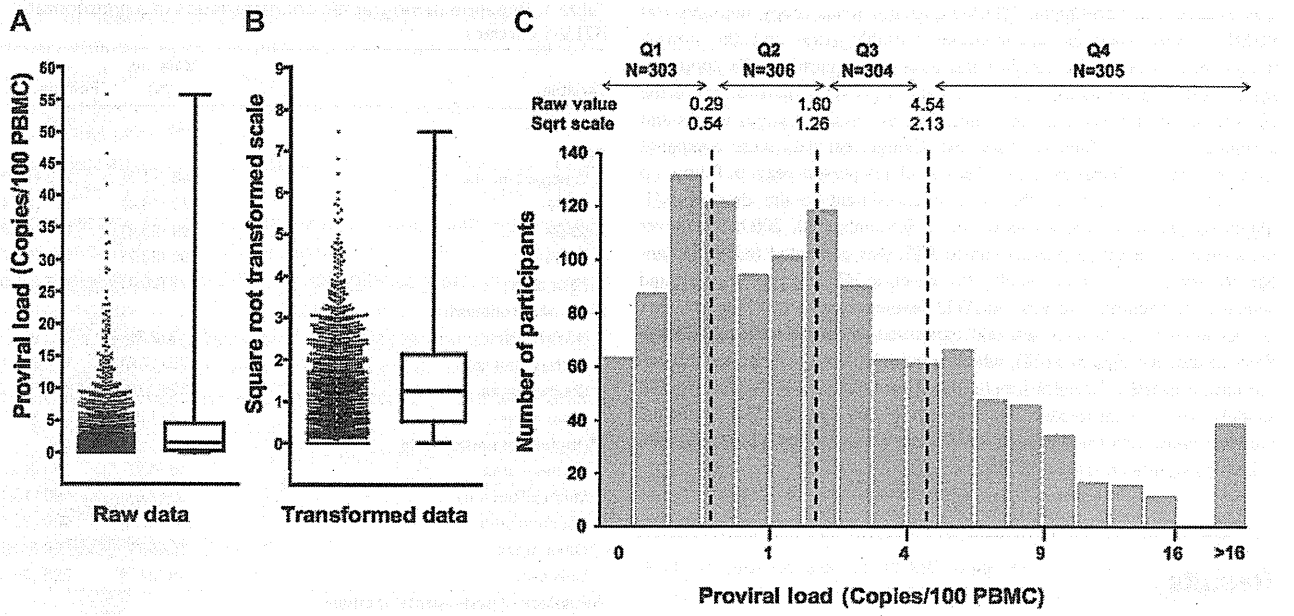

Figure 1. Distribution of baseline HTLV-1 proviral load levels among 1218 asymptomatic HTLV-1 carriers. (A) Scatter plot of raw data of proviral load (left) and the vertical box and whiskers plot (right): the box delineates 25th percentile (0.29 copies/100 peripheral blood mononuclear cells [PBMCs]), median (1.60 copies/100 PBMCs), and 75th percentiles (4.54 copies/100 PBMCs), and the whiskers delineate the minimum (0 copies/100 PBMCs) and maximum (55.8 copies/100 PBMCs). (B) Scatter plot of square-root transformed values of the raw proviral load (left) and the vertical box and whiskers plot (right): the box delineates 25th percentile (0.54 copies/100 PBMCs), median (1.26 copies/100 PBMCs), and 75th percentiles (2.13 copies/100 PBMCs), and the whiskers delineate the minimum (0 copies/100 PBMCs) and maximum (7.47 copies/100 PBMCs). (C) The frequency of participants in the quartile distributions of proviral load. Q1 indicates quartile 1 (< 25th percentile); Q2, quartile 2 (25th percentile to median); Q3, quartile 3 (median to 75th percentile); Q4: quartile 4 (> 75th percentile); Sqrt, square-root transformation; and N, number of participants.

PBMCs (range, 0-7.47 copies/100 PBMCs; 25th-75th percentile, 0.54-2.13 copies/100 PBMCs; Figure 1B). Figure 1C shows the frequency of participants in each quartile of proviral load.

The median proviral load and a frequency of subjects in each quartile of proviral load by demographic characteristics are shown in Table 2. Males and females were significantly different in proviral load levels, with a median value of 2.10 copies/100 PBMCs (range, 0-46.6 copies/100 PBMCs) for males and that of 1.39 copies/100 PBMCs (range, 0-55.8 copies/100 PBMCs) for females ($P < .001$). Males were probably distributed in the highest quartile of proviral load level than females.

Among age groups, the median proviral load of those 40 to 49 and 50 to 59 years of age was significantly higher than that of those less than or equal to 40 years ($P = .02$ and $P = .007$, respectively). Both age groups were probably distributed in the highest quartile of proviral load levels. Because we found a significantly different median proviral load by sex, we additionally evaluated the proviral load level by age group in each sex. The highest median value was found in those 50 to 59 years of age (2.89 copies/100 PBMCs) in males, but in 40 to 49 years of age (1.49 copies/100 PBMCs) in females, although there were no statistical differences by age group for both sexes (data not shown).

Among the categories for the first opportunity for HTLV-1 testing, the proviral load level was significantly higher ($P = .002$) in participants informed of their infection during treatment for diseases unrelated to HTLV-1 compared with those who came to know of their infection by screenings (Table 2). Participants informed of their infection during treatment for diseases unrelated to HTLV-1 were probably distributed in the highest quartile of proviral load levels. There was no difference in the proviral load level between those who came to know of their infection by the presence of HTLV-1–positive family members and those who came to know of their infection by screenings.

When we evaluated the proviral load level by family history status, participants who had no family history of HTLV-1 infection, who had only HTLV-1 carriers in the family, who had only an HTLV-1 carrier husband, and who had only HU/HAU in the family were grouped together as a reference category. The proviral load levels of those with a family history of HAM/TSP (median 3.85 copies/100 PBMCs) and ATL (median 2.32 copies/100 PBMCs) were significantly higher ($P = .01$ and $P = .005$, respectively) compared with those of the reference group (Table 2). Indeed, those with a family history of HAM/TSP and ATL were probably distributed in the third and fourth quartiles of proviral load levels. Of interest, the median proviral load level of those with a family history of leukemia or lymphoma was also significantly higher ($P = .009$) compared with those of the reference group.

Among the categories for comorbidity, there was no statistical difference in the proviral load levels when we simply compared between those with and without comorbidity at enrollment (data not shown). However, when we compared those without comorbidity and those with infectious diseases at enrollment, the median proviral load of the latter was significantly higher than that of the former ($P = .05$; Table 2).

Prognosis

During a median follow-up period of 1.0 year (range, 0-6.6 years) and a total of 1981.2 person-years, 14 (1.1%) participants (4 males and 10 females) progressed to overt ATL (2 acute, 2 lymphoma, and 10 smoldering types; Table 3). The incidence rate of ATL was 7.1 per 1000 person-years for all types of ATL and 2.0 per 1000 person-years for the aggressive types (acute and lymphoma) of ATL. The median duration from date of enrollment to date of diagnosis of ATL was 13.8 months (range, 2.8-64.4 months). The cumulative probability of progression to ATL was reached 4.8% (95% CI, 1.9%-11.8%) at 5.4 years (Figure 2).

Table 2. HTLV-1 VL levels by demographic characteristics

Demographic characteristics	No.	Median VL (range) (copies/100 PBMCs)	Frequency of subjects by VL level, n (% of row)			
			Quartile 1 (VL: < 0.29)‡	Quartile 2 (VL: 0.29-1.60)	Quartile 3 (VL: 1.60-4.54)	Quartile 4 (VL: ≥ 4.54)
Total		1.60 (0-55.8)	303	306	304	305
Sex						
Male	426	2.10 (0-46.6)*	84 (19.7)	100 (23.5)	93 (21.8)	149 (35.0)
Female	792	1.39 (0-55.8)†	219 (27.7)	206 (26.0)	211 (26.6)	156 (19.7)
Age, y						
Younger than 40	167	1.37 (0-16.4)†	49 (29.3)	43 (25.8)	50 (29.9)	25 (15.0)
40-49	200	1.77 (0-41.7)*	43 (21.5)	52 (26.0)	51 (25.5)	54 (27.0)
50-59	273	1.84 (0-36.1)*	64 (23.4)	64 (23.4)	63 (23.1)	82 (30.4)
60-69	260	1.56 (0-46.6)	66 (25.4)	66 (25.4)	61 (23.5)	67 (25.8)
70 or older	318	1.52 (0-55.8)	81 (25.5)	81 (25.5)	79 (24.8)	77 (24.2)
First opportunity for HTLV-1 testing						
Screening	661	1.46 (0-55.8)†	182 (27.5)	160 (24.2)	175 (26.5)	144 (21.8)
Revelation of HTLV-1–positive family	134	1.45 (0-46.6)	31 (23.1)	40 (29.9)	39 (29.1)	24 (17.9)
During treatment for other diseases	265	1.93 (0-41.7)*	56 (21.1)	66 (24.9)	57 (21.5)	86 (32.5)
Unknown	158	2.08 (0-30.3)*	34 (21.5)	40 (25.3)	33 (20.9)	51 (32.3)
Family history of HTLV-1–related diseases						
Absence or carrier/HU/HAU only	385	1.33 (0-32.4)†	100 (26.0)	105 (27.2)	100 (26.0)	80 (20.8)
HAM/TSP	9	3.85 (1.2-9.4)*	0	1 (11.1)	5 (55.6)	3 (33.3)
ATL	108	2.32 (0-46.6)*	18 (16.7)	26 (24.1)	33 (30.6)	31 (28.7)
Leukemia or lymphoma	35	2.47 (0-12.8)*	3 (8.6)	9 (25.7)	11 (31.4)	12 (34.3)
Unknown family history	681	1.55 (0-55.8)	182 (26.7)	165 (24.2)	155 (22.8)	179 (26.3)
Comorbidity						
Absence	961	1.65 (0-55.8)†	241 (25.1)	234 (24.4)	244 (25.4)	242 (25.2)
Infectious diseases	45	2.75 (0-28.6)*	7 (15.6)	8 (17.8)	13 (28.9)	17 (37.8)
Autoimmune diseases	29	1.33 (0-41.7)	10 (34.5)	7 (24.1)	4 (13.8)	8 (27.6)
Malignant diseases	80	1.57 (0-19.4)	19 (23.8)	21 (26.3)	23 (28.8)	17 (21.3)
Skin diseases	16	0.60 (0.07-14.6)	6 (37.5)	5 (31.3)	3 (18.8)	2 (12.5)
Other disease	87	1.17 (0-22.0)	20 (23.0)	31 (35.6)	17 (19.5)	19 (21.8)

HTLV-1 indicates human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; PBMCs, peripheral blood mononuclear cells; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; TSP, tropical spastic paraparesis; and ATL, adult T-cell leukemia.

*Mann-Whitney test revealed a statistically significant difference in the VL level compared with the reference group.

†Reference group.

‡The VL was categorized based on quartile cutoff points (the 25th, 50th, and 75th percentiles of the VL distribution) in 1218 HTLV-1 carriers. The unit of VL was copies/100 PBMCs.

The median proviral load at enrollment for these 14 participants was 10.3 copies/100 PBMCs (range, 4.17-28.58 copies/100 PBMCs), which was significantly higher than those who did not develop ATL (1.56 copies/100 PBMCs; range, 0-55.8 copies/100 PBMCs; $P < .001$). Of interest, the median proviral load level at enrollment was significantly higher for those who developed smoldering types of ATL than for those who developed aggressive types of ATL (11.4 and 5.1 copies/100 PBMCs, respectively, $P = .02$), whereas the median entry age was significantly younger for the former than for the latter (59.8 and 73.9 years, respectively, $P = .02$). Distribution of the 14 participants who developed ATL by demographic characteristics and by quartile of proviral load levels is shown in Table 4. Among 14 ATLs, 13 occurred in the highest quartile of baseline proviral load (> 4.54 copies/100 PBMCs) and 1 occurred in the third quartile (1.60-4.54 copies/100 PBMCs), whereas no ATL developed in quartiles 1 and 2 (< 1.60 copies/100 PBMCs). A high frequency of ATL was also seen in older age group, those with first opportunity for HTLV-1 testing during treatment of other diseases and those with a family history of ATL. Therefore, we decided to include the baseline HTLV-1 proviral load (the square-root transformed continuous value), age, first opportunity for HTLV-1 testing, and family history into Cox hazard analyses as covariates to test the effects on the development of ATL.

We identified that baseline proviral load was strongly associated with the risk of progression to ATL on both univariate and multivariate Cox analyses. In the multivariate analysis, the adjusted HR for the square-root transformed proviral load per unit increase was 3.57 (95% CI, 2.25-5.68; Table 5). We also found that advanced age, family history of ATL, and first opportunity to learn of HTLV-1 infection during treatment of other diseases were independently associated with the development of ATL, after adjusting the effect of proviral load. The adjusted HR for developing ATL per 5-year increase of age from 40 years was 1.67 (95% CI, 1.12-2.50). HTLV-1 carriers having a family history of ATL had 12 times higher risk of developing ATL compared with those not having the history (adjusted HR = 12.1; 95% CI, 2.26-64.7), and those who came to know their HTLV-1 infection during treatment for other diseases had 4 times higher risk of developing ATL compared with references (adjusted HR = 4.16; 95% CI, 1.37-12.6), although the CIs were wide because of the smaller group sizes (Table 5). Of interest, male gender was not a significant risk factor for developing ATL, even though the median proviral load was significantly higher in males than in females (Table 2).

Because the distribution of proviral load was skewed even after the value was square-root transformed, it was possible that ATL events in subjects with skewed high proviral loads contributed to results. To check the possibility, we performed a multivariate analysis using a sub-dataset that excluded subjects with skewed proviral load (> 16 copies in Figure 1C; n = 39, including 3 who developed ATL). Nevertheless, we observed similar results as the original dataset, although age factor was no longer statistically

Table 3. Cases who developed ATL from HTLV-I carrier status

	Demographic characteristics						Baseline clinical and biologic values					ATL development	
Case no.	Sex	Age, y	Place of birth	First opportunity for HTLV-1 testing	Family history of HTLV-1–related disease	Comorbidity at enrollment	HTLV-1 VL, copies/100 PBMCs	sIL-2R, U/mL	Abnormal lymphocytes, percentage	LDH, IU/L	WBC, $\times 10^3/mm^3$	Clinical type	Duration from enrollment, mo
Progression to aggressive type of ATL													
1	M	79.9	Southern	ATL family	ATL	None	5.47	479	2	157	4200	Acute	7.4
2	F	70.3	Southern	ATL family	ATL	None	4.73	904	0	365	9130	Acute	38.6
3	M	71.9	Southern	Other disease	None	Skin disease	4.17	1450	0	351	5140	Lymph	4.6
4	F	75.8	Southern	Unknown	Unknown	None	10.52	2080	3	308	3600	Lymph	30.6
Progression to indolent type of ATL													
5	F	60.0	Southern	Other disease	Unknown	None	9.12	340	14	192	5100	Sm	6.0
6	F	71.9	Southern	Multiphasic screening	None	None	10.60	1320	2	199	4000	Sm	29.8
7	F	59.5	Southern	Multiphasic screening	None	None	21.90	635	4.5	188	4100	Sm	12.0
8	F	74.0	Southern	Other disease	Unknown	Gallbladder cancer	10.11	1110	2	240	2700	Sm	26.8
9	F	54.1	Southern	Other disease	Unknown	None	18.85	971	2	198	5660	Sm	29.0
10	F	43.3	Southern	Pregnancy	ATL	None	13.90	372	1	ND	5400	Sm	64.4
11	F	62.2	Southern	Other disease	Unknown	Eye disease	6.86	1560	ND	508	12100	Sm	6.0
12	M	57.6	Southern	Other disease	Unknown	None	7.67	ND	2	234	5500	Sm	15.4
13	F	41.0	Metropolitan	Pregnancy	None	None	12.14	349	2.5	189	7690	Sm	12.2
14	M	66.1	Southern	Other disease	None	Prostatitis	28.58	2660	0	158	8500	Sm	2.8

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1;VL, HTLV-1 proviral load; PBMCs, peripheral blood mononuclear cells; sIL-2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; WBC, white blood cell count; Sm, smoldering type; and ND, not done.

significant ($P = .07$; supplemental Table 1). It is also possible that effects of some of the risk factors are weighted because of only 1 patient with an event because only 14 were analyzed as events in the multivariate analyses. To check the possibility, we performed 14 leave-one-out analyses, omitting 1 of 14 cases at a time from the original dataset. The Jackknifed coefficient of each parameter revealed the stability, which indicated that none of 14 cases affected the original model (data not shown).

Discussion

Previous studies reported no significant differences in the HTLV-1 proviral load by sex and age in asymptomatic HTLV-1 carriers.[21,22,24,33] In the present study, however, we found that there were significant differences in the proviral load by sex and age (Table 2). The median HTLV-1 proviral load was significantly higher in males than females. The median HTLV-1 proviral load for those 40 to 49 and 50 to 59 years of age was significantly higher than for those less than or equal to 40 years. The discrepancy between results of previous studies and those of the present study may be primarily explained by the differences in study population characteristics. We also found sex differences in age distributions of HTLV-1 proviral load; in male subjects, the median proviral load level was the highest at 50 to 59 years of age, whereas in female subjects it was highest at 40 to 49 years of age, although there were no statistical differences. These distribution characteristics of HTLV-1 proviral load are of interest when we consider the differences in sex and age at onset between ATL and HAM/TSP. ATL occurs predominantly in older males (~ 60 years), whereas HAM/TSP occurs predominantly in middle-aged females (~ 45-55 years). Thus, the proviral load levels of asymptomatic HTLV-1 carriers might be the highest in the age groups approximately 5 to 10 years before the average age at onset of ATL and HAM/TSP. These distribution characteristics may be related to differences in host immune responses to HTLV-1 and other unknown host factors.[34]

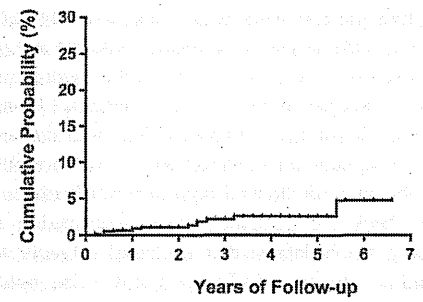

Figure 2. Probability of progression to ATL among 1218 asymptomatic HTLV-1 carriers.

The present study revealed that the median proviral load level of those with a family history of ATL or HAM/TSP was significantly higher than for those with no family history (Table 2). These results support previous studies indicating that HTLV-1–infected blood donors and asymptomatic carriers with familial HAM/TSP or ATL tend to have a higher HTLV-1 proviral load than those without family history.[21,33] In the present study, the proviral loads were also higher in those with a family history of leukemia or lymphoma than those without such history. We assume that the family history of leukemia or lymphoma may have included some ATL cases because some participants provided a diagnosis as just unknown leukemia or lymphoma. Although the present study was a large cohort, data collection regarding family history of HTLV-1–associated diseases was insufficient because one-half of the participants did not know their family HTLV-1 status. Further detailed data collection is needed to confirm the characteristics of HTLV-1 proviral load levels by family histories among asymptomatic HTLV-1 carriers, as this is necessary to determine genetic determinants of HTLV-1–associated diseases.

HTLV-1 carriers have various comorbidities, such as infectious, autoimmune, and malignant diseases.[4,25,35-38] In the present study, 45 participants had various infectious diseases at enrollment (Table

Table 4. Frequency of subjects who developed ATL by demographic characteristics and by VL level

Demographic characteristics	No. of subjects	No. of ATLs (% of subjects)	Frequency of ATL by VL level, n (% of subjects in each quartile in Table 2)		
			Quartiles 1 and 2 (VL: < 1.60)*	Quartile 3 (VL: 1.60-4.54)	Quartile 4 (VL: ≥ 4.54)
Total	1218	14 (1.1)	0	1 (0.3)	13 (4.3)
Sex					
Male	426	4 (0.9)	0	1 (1.1)	3 (2.0)
Female	792	10 (1.3)	0	0	10 (6.4)
Age, y					
Younger than 40	167	0	—	—	—
40-49	200	2 (1.0)	0	0	2 (3.7)
50-59	273	3 (1.1)	0	0	3 (3.7)
60-69	260	3 (1.2)	0	0	3 (4.5)
70 or older	318	6 (1.9)	0	1 (1.3)	5 (6.5)
First opportunity for HTLV-1 testing					
Screening	661	4 (0.6)	0	0	4 (2.8)
Revelation of HTLV-1-positive family	134	2 (1.5)	0	0	2 (8.3)
During treatment for other diseases	265	7 (2.6)	0	1 (1.8)	6 (7.0)
Unknown	158	1 (0.6)	0	0	1 (2.0)
Family history of HTLV-1–related diseases					
Absence or carrier/HU/HAU only	385	5 (1.3)	0	1 (1.0)	4 (5.0)
HAM/TSP	9	0	—	—	—
ATL	107	3 (2.8)	0	0	3 (9.7)
Leukemia or lymphoma	36	0	0	0	0
Unknown family history	681	6 (0.9)	0	0	6 (3.4)
Comorbidity					
Absence	961	10 (1.0)	0	0	10 (4.1)
Infectious diseases	45	1 (2.2)	0	0	1 (5.9)
Autoimmune diseases	29	0	—	—	—
Malignant diseases	80	1 (1.3)	0	0	1 (5.9)
Skin diseases	16	1 (6.3)	0	1 (33.3)	0
Other disease	87	1 (1.1)	0	0	1 (5.3)

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; TSP, tropical spastic paraparesis; and —, not applicable.

*The VL was categorized based on quartile cutoff points (the 25th, 50th, and 75th percentiles of the VL distribution) in 1218 HTLV-1 carriers. The unit of VL was copies/100 PBMCs.

1). We found that the median proviral load of these participants was significantly higher than that of those with no comorbidity (Table 2). The results of the present study support previous reports indicating higher HTLV-1 proviral loads in HTLV-1 carriers with comorbid *Strongyloides stercoralis* or bladder and kidney infections than those without such infections.[25,35,36] HTLV-1 carriers with rheumatoid arthritis or connective tissue disease and those with myelodysplastic syndromes carrying HLA-A26 were also reported to have higher HTLV-1 proviral loads compared with the median proviral load of those without such diseases.[37,38] In the present study, however, the median proviral load was not significantly high in those with autoimmune and malignant diseases. Further studies are required to find other predisposing factors affecting the proviral load level in each person.

A high HTLV-1 proviral load is currently considered as one of the main indicators for the progression to ATL.[20,28] In the present study, 14 participants of asymptomatic HTLV-1 carriers progressed to overt ATL as of 2009, all of whose baseline proviral load levels were high (range, 4.17-28.58 copies/100 PBMCs; Table 3). Therefore, we suggest that those with a high proviral load level (~ > 4 copies/100 PBMCs) are in a high-risk group for developing ATL (this group accounted for ~ 29% of the cohort). Multivariate Cox analyses confirmed that a higher proviral load level was a strong factor in the development of ATL (Table 5). This result strongly supports previous small-scale studies.[20,28] However, the role of the high proviral load level still remains unclear because the majority of asymptomatic carriers with a high HTLV-1 proviral load level in the present study remain carrier status. In the present study, male gender was not a significant risk factor for ATL, even though the median proviral load was significantly higher in males than in females. A high HTLV-1 proviral load is also reported to be associated with HAM/TSP.[20,21,27] These findings suggest that a high

Table 5. Cox proportional hazards modeling of risk factors for ATL development

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Male sex (vs female)	0.74(0.23-2.37)	.61	0.38(0.12-1.18)	.09
Square-root transformed VL per unit increase	2.55(1.91-3.41)	< .001	3.57(2.25-5.68)	< .001
Age per 5-year increase from 40 y	1.20(0.94-1.53)	.15	1.67(1.12-2.50)	.012
Family history of ATL (vs others)	2.68(0.80-8.98)	.11	12.1 (2.26-64.7)	.004
First opportunity for HTLV-1 testing during treatment of other diseases (vs others)	3.40(1.12-10.28)	.03	4.16(1.37-12.6)	.012

Analyses were performed using robust sandwich variance estimates.

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; HR, hazard ratio; and CI, confidence interval.

proviral load alone is not a unique predictive marker for ATL. In addition, the present study showed that the median proviral load level at enrollment was lower in those who developed aggressive types of ATL (5.1 copies/100 PBMCs) than that in those who developed smoldering types of ATL (11.4 copies/100 PBMCs; $P = .02$). This also suggests that a high proviral load alone is not a predictive marker for aggressive types of ATL.

In the present study, multivariate Cox analysis indicated that increased age, family history of ATL, and first opportunity to learn of HTLV-1 infection during treatment of other diseases were also independent risk factors for the development of ATL, after adjusting for proviral load (Table 5). This suggests that multiple risk factors (including unknown factors) are related to the progression from HTLV-1 carrier status to ATL. The reason why "opportunity to learn of HTLV-1 infection during treatment of other diseases" was an independent risk factor is unknown. The findings that more advanced states of HTLV-1 carriers (ie, an intermediate state[6] and a preleukemic state[13]) tend to be complicated by various comorbid diseases and that HTLV-1 carriers with various comorbid diseases had higher HTLV-1 proviral loads[25,35-38] could in part explain the reason.

Some prospective studies serially evaluated HTLV-1 proviral loads in HTLV-1 carriers and reported that their proviral load level was relatively stable over time with a certain level of fluctuations for persons.[25,26,28] Taylor et al reported that proviral loads of 20 HTLV-1 carriers were stable over a mean of 27 months, even though 9 carriers with various comorbidities showed high proviral load levels.[25] Meanwhile, an increasing proviral load was observed before progression to HAM/TSP and ATL.[27,28] However, there remain more questions how much of the fluctuations in proviral load over time could predict disease progression over the natural fluctuations within persons. Factors other than the proviral load level might be influencing the development of HTLV-1–associated diseases. Future studies should perform serial evaluations of HTLV-1 proviral loads by considering risk factors that have been confirmed in the present study.

The present study has several limitations. The number of ATL events was very small to obtain a conclusive result. However, we have a confidence for our results because we used a robust variance estimate in the multivariate analysis and because 2 validity analyses confirmed the original results. Data collection was insufficient for some items in the questionnaire. To resolve this issue, we will need to administer the questionnaire repeatedly. Our study design did not include enough information for evaluating the development of HAM/TSP. The follow-up duration is too short with regard to the natural history of ATL that has a long latency. Further follow-up of this cohort and similar prospective investigations should provide data needed to support more detailed conclusions. We did not compare the proviral loads by place of enrollment because we realized that many HTLV-1 carriers have migrated from the southern area to the metropolitan area.[39] The migration of HTLV-1 carriers has raised some public health issues in Japan. Screening for HTLV-1 in pregnant women and prevention programs for mother-to-child transmission of HTLV-1 are conducted in endemic areas[40,41] but not in metropolitan areas, which could introduce a higher chance of new HTLV-1 infections in the metropolitan area. To date, there is no nationwide program for preventing new HTLV-1 infections in Japan. Further nationwide studies are needed to determine the precise numbers of HTLV-1 carriers and to prevent HTLV-1 infection.

In conclusion, the present cohort study of 1218 asymptomatic HTLV-1 carriers provided detailed distributions for HTLV-1 proviral loads regarding the host-specific characteristics and the associations with the development of ATL. We confirmed that a higher proviral load levels (especially $\sim > 4$ copies/100 PBMCs), advanced age, family history of ATL, and having the first opportunity to learn of HTLV-1 infection during treatment of other diseases were independent risk factors for progression from carrier status to ATL. Further large-scale epidemiologic studies are needed to clearly identify the determinants of ATL for early detection and rapid cure for HTLV-1–associated diseases.

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Authorship

Contribution: M.I. managed the study database, analyzed data, and wrote the manuscript; T.W. organized the study and managed processing of the samples and measurement of proviral loads; A.U., A.O., K. Uchimaru, K.-R.K., M.O., H.K., K. Uozumi, M.M., K.T., Y. Saburi, M.Y., J.T., and Y.M. were responsible for participant enrollment and data collection; Y. Sagara managed the biomaterial bank; S.H. organized the study and managed the database; S.K. and K.Y. established the study; and all authors critically reviewed the article and approved the final version.

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A complete list of JSPFAD participants is available online in the supplemental Appendix.

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Long-term study of indolent adult T-cell leukemia-lymphoma

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The long-term prognosis of indolent adult T-cell leukemia-lymphoma (ATL) is not clearly elucidated. From 1974 to 2003, newly diagnosed indolent ATL in 90 patients (65 chronic type and 25 smoldering type) was analyzed. The median survival time was 4.1 years; 12 patients remained alive for more than 10 years, 44 progressed to acute ATL, and 63 patients died. The estimated 5-, 10-, and 15-year survival rates were 47.2%, 25.4%, and 14.1%, respectively, with no plateau in the survival curve. Although most patients were treated with watchful waiting, 12 patients were treated with chemotherapy. Kaplan-Meier analyses showed that advanced performance status (PS), neutrophilia, high concentration of lactate dehydrogenase, more than 3 extranodal lesions, more than 4 total involved lesions, and receiving chemotherapy were unfavorable prognostic factors for survival. Multivariate Cox analysis showed that advanced PS was a borderline significant independent factor in poor survival (hazard ratio, 2.1, 95% confidence interval, 1.0-4.6; $P = .06$), but it was not a factor when analysis was limited to patients who had not received chemotherapy. The prognosis of indolent ATL in this study was poorer than expected. These findings suggest that even patients with indolent ATL should be carefully observed in clinical practice. Further studies are required to develop treatments for indolent ATL. (*Blood.* 2010;115(22):4337-4343)

Introduction

Adult T-cell leukemia-lymphoma (ATL) is a peripheral T-lymphocytic malignancy associated with human T-cell lymphotropic virus type 1 (HTLV-1).[1] ATL has been classified into 4 clinical subtypes: acute, lymphoma, chronic, and smoldering.[2] In general, acute and lymphoma types of ATL have a extremely poor prognosis despite advances in chemotherapy and allogenic hematopoietic stem cell transplantation[3-5] because of multidrug resistance, a large tumor burden with multiorgan failure, hypercalcemia, and/or frequent infectious complications associated with a T-cell immunodeficiency. A previous study, in which Japanese patients with ATL were followed for a maximum duration of 7 years, reported that the 4-year survival rates for acute, lymphoma, chronic, and smoldering type were 5.0%, 5.7%, 26.9%, and 62.8%, respectively, with the median survival time (MST) of 6.2 months, 10.2 months, 24.3 months, and not yet reached, respectively.[2] Therefore, the chronic and smoldering subtypes of ATL are considered indolent and are usually managed with watchful waiting until disease progression to acute crisis, similar to the management of chronic lymphoid leukemia or smoldering myeloma. However, the follow-up duration of the previous Japanese study was too short for indolent ATL to evaluate the overall risk of progression to acute or lymphoma types (ie, aggressive ATL). A recent Brazilian study, in which patients with ATL were followed for a maximum duration of 14 years, reported that the MST of chronic and smoldering types were 18 months and 58 months, respectively, and the overall survival (OS) rates were less than 20% in both types.[6] Their results suggest that the long-term prognosis of indolent ATL might be worse than expected.

The long-term prognosis of Japanese patients with indolent ATL has not been well evaluated so far. Prognostic factors for patients with indolent ATL are also unclear. In the present study, we investigated the long-term outcome of 90 patients with indolent ATL. The purposes of this study were to estimate the 5-, 10-, and 15-year survival rates for indolent ATL and to evaluate the prognostic factors.

Methods

Patients

We evaluated a total of 90 patients with indolent ATL (25 smoldering type and 65 chronic type) who were newly diagnosed at the Nagasaki University Hospital between July 1974 and December 2003. The distribution of patients by year of diagnosis in decades (1974-1983, 1984-1993, and 1994-2003) are presented in Table 1. The cutoff date for analysis was December 2008. The diagnosis of ATL was based on clinical features, histologically and/or cytologically proven mature T-cell malignancy, the presence of anti–HTLV-1 antibody, and monoclonal integration of HTLV-1 proviral DNA into tumor cells as described previously.[2,7-9] The subtypes of ATL were classified according to criteria established by the Lymphoma Study Group of Japan Clinical Oncology Group.[2] Clinical data included date of diagnosis, complications at diagnosis, therapy regimens if applicable, date of death, cause of death, and date of latest contact. This retrospective, nonrandomized, observational study that used existing data

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Table 1. Distribution of patients in 3 decades from 1974 to 2003

Year	Total no. of patients	No. of smoldering type (% of total)	No. of chronic type (% of total)
1974-1983	19	2 (10.5)	17 (89.5)
1984-1993	35	7 (20.0)	28 (80.0)
1994-2003	36	16 (44.4)	20 (55.6)
Total for all years	90	25 (27.8)	65 (72.2)

was granted an exemption from the institutional review board and waived the requirement for written informed consent.

Clinical factors and definitions

Age was dichotomized into 2 groups: 60 years or older and younger than 60 years. Performance status (PS) was based on the 5-grade scale of the World Health Organization. Complications at diagnosis were dichotomized into present and absent. Leukocytosis was defined as white blood cell count of 12×10^9/L or greater with the median value as cutoff level. Lymphocytosis was defined as a total lymphocyte count of 6.5×10^9/L or greater with the median value as cutoff level. Neutrophilia was defined as a neutrophil count of 7.5×10^9/L or greater.[10] Eosinophilia was defined as an eosinophil count of 0.4×10^9/L greater.[11] Lactate dehydrogenase (LDH) and blood urea nitrogen (BUN) were dichotomized into normal and elevated concentrations.[12] Albumin was dichotomized into concentrations of 40.0 g/L (4.0 g/dL) or greater and less than 40.0 g/L (4.0 g/dL).[2] Potential prognostic factors (PPFs) for chronic ATL were defined as those with at least one of the following 3 factors: low serum albumin, high LDH, or high BUN according to previous reports.[13,14] Tumor lesions were evaluated as the number of lymph node lesions, number of extranodal lesions, and number of total involved lesions. Extranodal lesions were defined as follows: bone marrow (BM) involvement as the presence of more than 5% typical ATL cells on a BM smear or detection of their infiltration in a BM biopsy specimen; skin involvement as the presence of ATL infiltration in a skin biopsy specimen or as the clinically presence of typical types of skin lesions such as tumors, nodules, erythema, and papules, if biopsy was impossible; lung involvement as lesions with ATL cell infiltration in a transbronchial lung biopsy specimen or in bronchoalveolar lavage fluid; liver involvement as hepatomegaly determined by any imaging tests or liver biopsy if done; spleen involvement as splenomegaly on any imaging test. All patients had peripheral blood involvement. Both lymph node and extranodal tumor lesions were determined according to Ann Arbor classification.[2] The number of total involved lesions was defined as the sum of lymph node lesions and extranodal lesions.[2] Factors used in analyses were listed in Table 2.

Statistical analysis

OS was defined as the time from the date of first diagnosis to the date of death or the latest contact with the patient. Survival curves were estimated using the Kaplan-Meier method and were compared using the generalized Wilcoxon test. MST was estimated as the time point at which the Kaplan-Meier survival curves crossed 50%. Time to transformation was calculated as the time from the date of the first diagnosis to the date of transformation into the aggressive type (acute or lymphoma type). Univariate and multivariate Cox regression analyses were applied to evaluate prognostic factors for survival. The effects of clinical parameters were evaluated as hazard ratios (HRs) and their 95% confidence intervals (95% CIs). All statistical analyses were performed using SAS software (Version 9.1; SAS Japan Institute). All tests were 2-tailed, and the statistical significance level was set at .05.

Results

Baseline characteristics

The median value of white blood cell count, lymphocyte count, neutrophil count, and eosinophil count was 11.5×10^9/L (range, 3.9-94.4 $\times 10^9$/L), 6.5×10^9/L (range, 0.9-80.2 $\times 10^9$/L), 4.9×10^9/L (range, 1.5-25.5 $\times 10^9$/L), and 0.06×10^9/L (range, 0-3.0 $\times 10^9$/L), respectively. Frequencies of the patients at baseline are summarized in Table 2. Fifty-eight percent of the patients were male, 52% were 60 years or older, and 22% had an advanced PS (2 or more grade). Regarding complications, 35 patients (39%) had some complications at the time of diagnosis, including 13 with chronic pulmonary diseases (10 chronic bronchitis, 2 diffuse panbronchiolitis, and 1 bronchial asthma with chronic bronchitis), 9 with opportunistic infections (3 pneumocystis pneumonia, 2 cryptococcal meningitis, 2 aspergillus pneumonia, 1 cytomegalovirus infection, and 1 pulmonary tuberculosis), 7 with malignancies other than ATL (2 lung cancer, 1 larynx cancer, 1 pharynx cancer, 1 colon cancer, 1 hepatic cell carcinoma, and 1 lip cancer), and 6 with autoimmune diseases (2 infective dermatitis, 1 primary biliary cirrhosis, 1 autoimmune hemolytic anemia, 1 dermatomyositis, and 1 ulcerative colitis). The 6 patients with autoimmune diseases had received a variety of medications as follows: antibiotics for infective dermatitis, ursodeoxycholic acid for primary biliary cirrhosis, prednisolone for autoimmune hemolytic anemia and dermatomyositis, and sulfasalazine for ulcerative colitis. Concerning the hematologic factors, 43 patients (48%) had leukocytosis, 45 (50%) had lymphocytosis, 17 (19%) had neutrophilia, and 17 (19%) had eosinophilia. Regarding the laboratory factors, 28 patients (31%) had a high LDH level (greater than the normal limit). Only 5 of 87 patients (6%) had an abnormal BUN level; 34 of 88 patients (39%) had a low albumin level. Forty-seven patients (55%) had more than 1 of the 3 unfavorable prognostic factors.

Twenty-four patients (27%) had more than 2 involved lymph node lesions. Regarding the extranodal lesions, skin involvement was observed in 46 patients (51%), liver involvement in 15 (17%), spleen involvement in 6 (7%), and pulmonary involvement in 1 (1%). Of the 64 patients who had BM examined, the involvement was observed in 16 patients (25%; data not shown). Twenty percent of the patients (n = 18) had more than 3 extranodal lesions. Regarding the number of total involved lesions (extranodal lesions plus lymph node lesions), more than 4 involved lesions were observed in 24 patients (27%), 2 or 3 involved lesions in 42 patients (46%), and only 1 involved lesion in 24 patients (27%).

Prognosis

Among 90 patients with indolent ATL, 63 (70%) died, with a median duration of follow-up of 4.1 years (range, 8 days to 17.6 years). The estimated 5-, 10-, and 15-year survival rates were 47.2% (95% CI, 36.1%-57.5%), 25.4% (95% CI, 15.3%-36.8%), and 14.1% (95% CI, 6.2%-25.3%), respectively, with an MST of 4.1 years (95% CI, 2.9-6.3 years; Figure 1A). No plateaus were observed in the survival curves for OS. Of the 27 survivors, 12 were alive for more than 10 years. Of the 63 patients who died, 41 (65.1%) died of acute ATL after transformation, 5 (7.9%) died of severe chronic ATL, 11 (17.5%) died of other diseases (3 malignancies other than ATL, 2 chronic pulmonary diseases, 2 opportunistic infections, 2 autoimmune diseases, 1 cardiac failure, and 1 myocardial infarction), 2 died of transplantation-related complications, and 4 died of unknown cause. No significant difference in OS was observed between patients who died of ATL and patients who died of other causes (data not shown). Among 90 patients, 44 (49%) progressed to aggressive ATL (all were acute types), among those, 41 (93%) died. The median time to transformation was 18.8 months (range, 0.3 months to 17.6 years).

Table 2. Survival by baseline clinical factors

Factors	No. of evaluated (% of total)	No. of deaths (%)*	MST, y	Cumulative probability of survival†		
				5-y survival, % (95% CI)	10-y survival, % (95% CI)	P†
Total	90	63 (70)	4.1	47.2 (36.1-57.5)	25.4 (15.3-36.8)	
Clinical subtype						
Smoldering	25 (28)	17 (68)	2.9	39.4 (19.8-58.6)	25.3 (8.2-47.0)	.36
Chronic	65 (72)	46 (71)	5.3	50.2 (37.0-62.0)	26.3 (14.6-39.5)	
Patient-related factors (n = 90)						
Sex						
Male	52 (58)	34 (65)	4.3	48.1 (33.4-61.3)	24.9 (11.8-40.5)	.99
Female	38 (42)	29 (76)	4.1	46.4 (29.5-61.6)	26.5 (12.0-43.4)	
Age						
60 y or older	46 (52)	32 (70)	3.7	45.5 (30.4-59.4)	29.5 (14.8-45.8)	.18
Younger than 60 y	44 (48)	31 (70)	4.5	49.2 (32.9-63.6)	24.0 (11.2-39.3)	
PS						
0	22 (24)	15 (68)	8.4	75.9 (51.4-89.1)	38.9 (16.8-60.7)	.006
1	49 (54)	33 (67)	3.4	41.5 (26.9-55.5)	22.5 (9.7-38.5)	
2 or 3	19 (22)	15 (79)	1.3	27.9 (10.2-49.0)	13.9 (1.3-41.1)	
Complications at diagnosis (n = 90)						
Absent	55 (61)	37 (67)	5.7	54.1 (39.4-66.7)	25.4 (12.9-40.1)	
Present	35 (39)	26 (74)	3.4	36.6 (20.7-52.8)	28.3 (13.5-45.1)	.06
Malignancies other than ATL	7 (8)	6 (86)	0.8	28.6 (4.1-61.2)	28.6 (4.1-61.2)	
Opportunistic infection	9 (10)	7 (78)	1.2	0	0	
Chronic pulmonary disease	13 (14)	10 (77)	4.1	38.5 (14.1-62.8)	25.6 (5.2-53.4)	
Autoimmune disease	6 (7)	3 (50)	11.4	62.5 (14.2-89.3)	62.5 (14.2-89.3)	
Hematologic factors						
WBC count (n = 90)						
At least 12.0 × 10^9/L	43 (48)	32 (74)	3.4	43.0 (27.6-57.5)	22.3 (9.9-37.8)	.24
Less than 12.0 × 10^9/L	47 (52)	31 (66)	5.3	51.0 (35.1-64.8)	28.5 (13.6-45.2)	
Total lymphocyte count (n = 90)						
At least 6.5 × 10^9/L	45 (50)	35 (78)	3.7	43.3 (28.2-57.5)	17.4 (6.8-32.0)	.34
Less than 6.5 × 10^9/L	45 (50)	28 (62)	5.3	51.4 (35.2-65.4)	36.8 (20.9-52.9)	
Neutrophil counts (n = 89)						
At least 7.5 × 10^9/L	17 (19)	14 (82)	2.3	29.4 (10.7-51.1)	14.7 (1.3-42.9)	.05
Less than 7.5 × 10^9/L	72 (81)	48 (67)	5.3	51.0 (38.3-62.4)	28.4 (16.6-41.3)	
Eosinophil count (n = 89)						
At least 0.4 × 10^9/L	17 (19)	11 (65)	4.0	34.9 (13.0-58.0)	23.2 (4.9-49.4)	.47
Less than 0.4 × 10^9/L	72 (81)	51 (71)	4.5	49.2 (36.8-60.5)	27.4 (16.0-40.1)	
Laboratory factors						
LDH (n = 90)						
Greater than NI	28 (31)	23 (82)	1.5	34.8 (17.3-53.0)	14.9 (3.9-32.7)	.004
Less than or equal to NI	62 (69)	40 (65)	5.4	52.9 (39.2-64.8)	31.8 (18.5-45.9)	
BUN (n = 87)						
Greater than NI	5 (6)	5 (100)	2.0	20.0 (0.8-58.2)	0	.18
Less than or equal to NI	82 (94)	56 (68)	4.5	48.9 (37.2-59.6)	28.4 (17.3-40.6)	
Albumin (n = 88)						
Less than 40.0 g/L	34 (39)	22 (65)	3.4	39.9 (22.4-56.8)	25.6 (8.9-46.4)	.22
At least 40.0 g/L	54 (61)	40 (74)	5.3	52.2 (37.9-64.7)	26.6 (14.3-40.6)	
Potential prognostic factors (n = 87)‡						
At least 1	47 (55)	34 (72)	2.9	38.7 (24.1-53.1)	18.1 (6.5-34.3)	.05
None	40 (45)	27 (68)	5.4	56.1 (39.2-70.0)	35.2 (19.3-51.6)	
Tumor lesions (n = 90)						
No. of lymph node lesions						
2 or more	24 (27)	16 (67)	2.1	37.5 (19.0-56.0)	30.0 (12.1-50.4)	.09
0 or 1	66 (73)	47 (71)	5.3	50.9 (37.5-62.8)	23.6 (12.2-37.2)	
No. of extranodal lesions						
3 or more	18 (20)	14 (78)	1.1	29.4 (10.7-51.1)	19.6 (4.2-43.3)	.005
1 or 2	72 (80)	49 (68)	5.3	51.6 (38.9-62.9)	26.8 (15.2-39.7)	
No. of total involved lesions						
4 or more	24 (27)	16 (67)	1.3	34.8 (16.6-53.7)	26.1 (8.8-47.6)	.03
2 or 3	42 (46)	30 (71)	4.5	49.5 (32.7-64.3)	13.1 (3.5-29.1)	
1	24 (27)	17 (71)	5.4	54.5 (32.1-72.4)	44.1 (22.8-63.5)	
Chemotherapy						
Received	12 (13)	12 (100)	1.4	25.0 (6.0-50.5)	0	.01
Not received	78 (87)	51 (65)	5.3	50.8 (38.6-61.8)	31.3 (19.3-44.0)	

WBC indocates white blood cell count; MST, median survival time (years); and NI, normal index.

*Rate of death in evaluated cases.

†Cumulative probability of survival rate was estimated with the Kaplan-Meier method, and the *P* value was calculated with the generalized Wilcoxon test.

‡PPFs indicate at least 1 of the following 3 factors: low serum albumin, high LDH, or high BUN.[13,14]

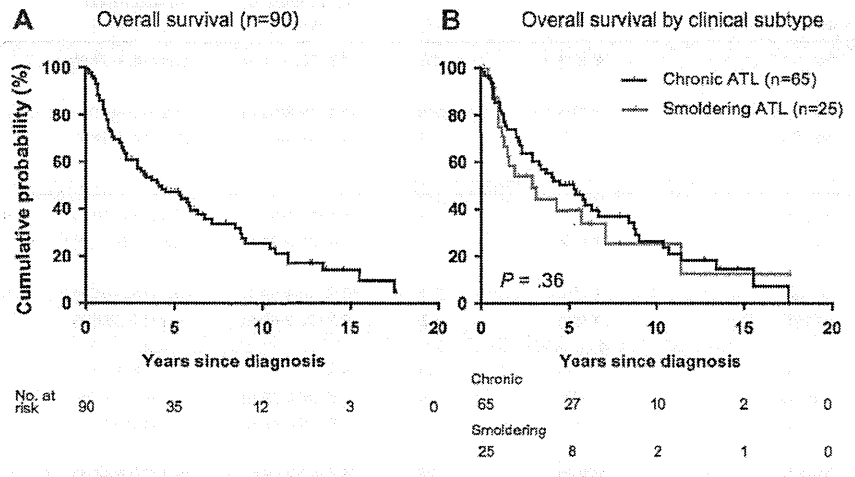

Figure 1. Survival of patients with indolent ATL. (A) For OS (n = 90), the median survival time was 4.1 years (95% CI, 2.9-6.3 years). No plateau was observed in the survival curves for OS. The estimated 5-, 10-, and 15-year survival rates were 47.2% (95% CI, 36.1%-57.5%), 25.4% (95% CI, 15.3%-36.8%), and 14.1% (95% CI, 6.2%-25.3%), respectively. (B) OS by clinical subtype (smoldering type vs chronic type). The estimated 15-year survival rate was 12.7% (95% CI, 1.1%-38.8%) with an MST of 2.9 years for smoldering type and 14.7% (95% CI, 5.7%-27.8%) with an MST of 5.3 years. There was no statistically significant difference ($P = .36$).

Among 25 patients with smoldering ATL, 17 patients (68%) died, and the estimated 15-year survival rate was 12.7% (95% CI, 1.1%-38.8%) with an MST of 2.9 years (95% CI, 1.3-7.1 years). Of the 17 patients who died, 15 died of acute ATL after transformation. Among 65 patients with chronic ATL, 46 (71%) died, and the estimated 15-year survival rate was 14.7% (95% CI, 5.7%-27.8%) with an MST of 5.3 years (95% CI, 2.9-6.7 years). Of the 46 patients who died, 29 died of acute ATL after transformation and 5 died of the disease severity. No statistically significant difference was observed in OS between subtypes ($P = .36$; Figure 1B). The overall estimated 5- and 10-year survival rates of both subtypes are shown in Table 2.

Effects of clinical factors on prognosis

Effects of clinical factors on prognosis were analyzed with the use of all the 90 patients together. Results of prognostic analyses (estimated 5- and 10-year OS rates and MST) with the use of Kaplan-Meier methods are summarized in Table 2. The survival rate was poor for patients with advanced PS ($P = .006$; Figure 2A), neutrophilia ($P = .05$; Figure 2B), and a higher LDH level ($P = .004$; Figure 2C). Patients with at least 1 of 3 PPFs for chronic ATL (a high level of LDH and BUN and a low level of albumin)[13,14] showed a poor survival rate compared with patients without ($P = .05$; Figure 2D). The difference in survival rates between patients with any complications and patients without was marginally significant ($P = .06$). Among patients with any complications, those with malignancies other than ATL or opportunistic infections at diagnosis showed a tendency of poor prognosis, although the number of patients in each category was too small (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Although no difference was observed in survival rates between patients with involvement of more than 2 lymph node lesions and patients with less involvement ($P = .09$; Table 2), the survival rate of patients

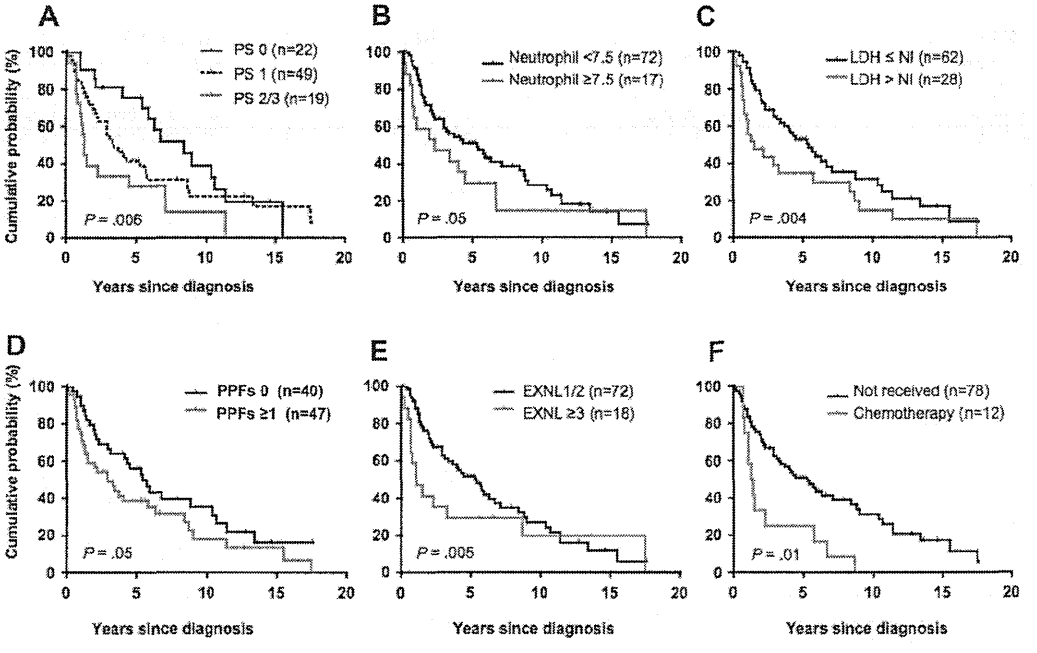

Figure 2. OS by clinical parameters. (A) OS by PS ($P = .006$). (B) OS by neutrophil count ($P = .05$). The unit is $\times 10^9$/L. (C) OS by LDH level ($P = .004$). NI indicates normal index. (D) OS by PPFs for chronic ATL that were defined based on low serum albumin, high LDH, or high BUN according to previous reports[13,14] ($P = .05$). (E) OS by the number of extranodal lesions (EXNL; $P = .005$). (F) OS by treatment states ($P = .01$).

Table 3. Effects of clinical factors on OS in Cox analyses

	All patients (n = 90)						Patients had not received chemotherapy (n = 78)					
	Univariate analysis		Multivariate model A		Multivariate model B		Univariate analysis		Multivariate model C		Multivariate model D	
Clinical factor	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
PS												
0	1		1		1		1		1		1	
1	1.5 (0.8-2.7)	.22	1.4 (0.8-2.8)	.27	1.3 (0.7-2.6)	.37	1.4 (0.7-2.7)	.28	1.6 (0.8-3.1)	.21	1.4 (0.7-2.9)	.30
2 or more	2.5 (1.2-5.2)	.01	2.1 (1.0-4.6)	.06	2.1 (1.0-4.6)	.06	1.7 (0.7-4.0)	.26	1.5 (0.6-3.8)	.39	1.6 (0.6-4.2)	.30
Neutrophil counts												
Less than 7.5×10^9/L	1		1		1		1		1		1	
7.5×10^9/L or greater	1.6 (0.9-2.9)	.15	1.3 (0.6-2.7)	.45	1.2 (0.6-2.3)	.58	1.3 (0.6-2.7)	.47	1.5 (0.6-3.8)	.43	1.0 (0.5-2.3)	.94
LDH												
Less than or equal to NI	1		1		1		1		1		1	
Greater than NI	1.7 (1.0-2.9)	.04	1.5 (0.8-2.7)	.16	1.5 (0.8-2.6)	.21	1.5 (0.8-2.8)	.19	1.7 (0.9-3.3)	.12	1.6 (0.8-3.1)	.20
No. of extranodal lesions												
0-2	1		1				1		1			
3 or more	1.5 (0.8-2.8)	.16	0.7 (0.3-1.6)	.41			0.9 (0.4-2.2)	.82	0.5 (0.1-1.6)	.22		
No. of total involved lesions												
1	1				1		1				1	
2 or 3	1.2 (0.7-2.2)	.52			0.8 (0.4-1.6)	.52	1.1 (0.6-2.1)	.67			0.9 (0.4-1.7)	.65
4 or more	1.5 (0.7-3.0)	.26			0.9 (0.4-2.1)	.83	1.0 (0.5-2.3)	.96			0.8 (0.3-2.0)	.67
Chemotherapy												
Not received	1		1		1							
Received	2.6 (1.4-5.1)	.003	2.3 (1.1-4.7)	.03	2.0 (1.0-4.2)	.06						

HR indicates hazard ratio; 95% CI, 95% confidence interval; and NI, normal index.

with more than 3 extranodal lesions was significantly poor than the others ($P = .005$; Figure 2E). The survival rate was worse in patients with more than 4 total involvement lesions than in the others (Table 2). Of the extranodal lesions, we additionally examined the effect of skin lesion and BM involvement on survival rates. The survival rate of patients with BM involvement was significantly poor than of patients without ($P = .04$; data not shown), but that of patients with skin involvement was not different from those without ($P = .66$; supplemental Figure 2).

Although most patients in this study had not been treated until their disease progression was similar to B-cell chronic lymphoid leukemia, 12 patients with chronic ATL were treated with chemotherapy immediately after diagnosis because of elevated LDH levels in 8 patients, severe BM involvement in 2 patients, and severe skin involvements in 2 patients. Among them, 2 patients were treated with VCAP (vincristine, cyclophosphamide, doxorubicin, and prednisone)–AMP (doxorubicin, ranimustine, and prednisone)–VECP (vindesine, etoposide, carboplatin, and prednisone),[3] 2 with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), 4 with CHOP-like, 3 with VEPA (vincristine, etoposide, prednisone, and doxorubicin),[15] and 1 with low-dose etoposide. All of these patients died (MST, 1.4 years; 95% CI, 1.1-2.3 years), and their prognosis was very poor compared with patients not treated ($P = .01$; Figure 2F).

On the basis of results from Kaplan-Meier curves and univariate analysis for each factor, we decided to include PS category, dichotomized neutrophil counts, dichotomized LDH category, dichotomized number of extranodal lesions, the number of total involved lesions, and chemotherapy states into multivariate Cox analysis. Model A included PS category, dichotomized neutrophil counts, dichotomized LDH category, dichotomized number of extranodal lesions, and chemotherapy states. Model B included the same factors as model A except for the number of total involved lesions instead of the number of extranodal lesions. This was because, by definition, a factor of the number of total involved lesions included a factor of the number of extranodal lesions. Results were summarized in Table 3. In model A, advanced PS (≥ 2; HR, 2.1; 95% CI, 1.0-4.6; $P = .06$, borderline significance) and chemotherapy states (HR, 2.3; 95% CI, 1.1-4.7; $P = .03$, significance) were correlated with OS, but the remaining factors were not independent prognostic factors after adjustment for covariate factors. To evaluate effects of clinical factors beyond the effect of chemotherapy states on OS, we also performed additional multivariate analyses for patients who were not received chemotherapy (n = 78; model C and model D in Table 3). We found that there was no clinical parameter that associated with OS.

Discussion

In the present study, we investigated for the first time the long-term clinical course of patients with indolent ATL with a maximum duration of follow-up of 17.6 years. We found that the prognosis of indolent ATL was poor with the MST of 4.1 years, and the estimated 15-year OS rates were 14.1% with no plateau in the survival curve. The prognosis observed in the present study was poorer than expected. Our results confirmed a recent long-term Brazilian study,[6] that showed a poor OS of less than 20% for indolent ATL. In the present study, we showed that 65.1% of patients died of acute ATL with a median time to transformation of 18.8 months. This finding suggests that most patients with indolent ATL will eventually die of aggressive ATL during their long-term course of illness. These findings suggest that even patients with indolent ATL should be carefully observed by frequent clinical visits.

The cause of death in patients with indolent ATL has not been well reported so far. In the present study, patients with indolent ATL died of various causes such as malignancies other than ATL,

chronic pulmonary diseases, opportunistic infections, and autoimmune diseases, in addition to death from acute ATL after transformation. A previous long-term study, which followed-up 50 HTLV-1 carriers with monoclonal proliferation of T lymphocytes (pre-ATL) for 20 years, also reported that 10 patients died of opportunistic infections such as *Pneumocystis* pneumonia or malignancies other than ATL (skin carcinoma, lung cancer, etc).[16] Patients with indolent ATL were also comorbid with a variety of diseases at diagnosis such as chronic pulmonary disease, opportunistic infections, multiple cancers, and autoimmune diseases in the present study. The pathogens responsible for the opportunistic infections were similar to those observed in patients with AIDS associated with HIV. Opportunistic infection was previously reported as a frequent complication in patients with aggressive or with indolent ATL.[2] These findings suggest that helper T-cell function in indolent ATL might be impaired similar to that in AIDS.[17]

We also presented that chronic pulmonary disease, multiple cancers, and autoimmune diseases were frequent as complications at diagnosis in indolent ATL. The reason why indolent ATL had such immune dysregulation remains unknown. It was recently noted that the origin of the ATL cells in a fraction of the patients was from regulatory T cells expressing FoxP3 and CCR4.[18,19] In the present study, 6 patients also had autoimmune diseases. Among them, 3 patients were treated with immunosuppressive drugs, and of those only one patient with smoldering ATL transformed to acute ATL. Therefore, we were not able to evaluate the effect of comorbid autoimmune diseases and immunosuppressive drug therapy on the risk of transformation or poor prognosis so far. Further studies are warranted to elucidate the mechanisms responsible for the development of hyperimmunity or hypoimmunity in patients with indolent ATL.

Although comparison on OS by subtype is not a primary purpose of this study, it was unexpected that survival rates of smoldering ATL (15-year OS, 12.7%) tended to be lower than chronic ATL (15-year OS, 14.7%), and the MST of smoldering ATL (2.9 years) tended to be shorter than chronic ATL (5.3 years; Table 2; Figure 1B). Transformation rates of smoldering ATL and chronic ATL were 60% (n = 15) and 44% (n = 29), respectively (data not shown), which was also unexpected. Although there was no statistically difference in OS, MST, and transformation rate between the 2 groups, our results were different from a previous short-time follow-up study reported by Shimoyama et al[2] (the 4-year survival rates for smoldering type was 62.8%). It was unknown why the rate of smoldering type was poorer than chronic type in the present study. Some previous studies suggested that skin involvements might be a risk factor for poor prognosis of smoldering ATL.[6,20-22] In the present study, the frequency of patients with skin lesion was a little higher in smoldering ATL (n = 14; 56%) than in chronic ATL (n = 32; 49%). The OS of smoldering ATL with skin lesion was worse than that of chronic ATL without skin lesion (supplemental Figure 2), although there was no statistical difference ($P = .5$). Therefore, a possible explanation might be that smoldering ATL with poor conditions (eg, skin involvement) might be disproportionately included in the present study because data were collected at a university hospital, where more advanced cases were referred from city clinics. Another possible explanation might be that the percentage of patients with smoldering-type ATL has increased recently, as shown in Table 1. In recent decades, more patients have been diagnosed with the smoldering type of ATL on the basis of a health examination, including a blood cell count. Some of these patients may have been in the early phase of acute ATL.

Shimoyama et al[2] reported that involved lymph node lesions, extranodal lesions, and total involvement lesions were significantly poor prognostic factors for ATL all together, and low serum albumin, high LDH, or high BUN levels were PPFs for chronic ATL.[13,14] As we expected, patients with at least 1 of 3 known PPFs for chronic ATL (a high level of LDH and BUN and a low level of albumin)[13,14] showed a poor survival rate than patients without (Table 2; Figure 2D). We also confirmed the difference was seen when analyses were performed for chronic ATL only ($P = .03$) but was not seen for smoldering ATL only ($P = .62$; supplemental Figure 3). This suggests that there may be different prognostic factors for smoldering ATL and chronic ATL, respectively. Further detailed studies regarding prognostic factors are needed for individual subtype.

Other than the known 3 potential prognostic factors, an advanced PS, neutrophilia, more than 3 extranodal lesions, more than 4 total involved lesions, and having received chemotherapy were shown to be possible unfavorable prognostic factors for indolent ATL in our Kaplan-Meier analyses (Table 2; Figures 1B, 2A-F). However, in multivariate Cox analyses, only advanced PS and chemotherapy state were associated with OS after adjustment for other covariates (models A and B in Table 3). The poor prognosis in patients with indolent ATL who were treated by chemotherapy was similar to that of the patients with unfavorable chronic ATL who were treated with intensive combination chemotherapy in several clinical trials in Japan.[3,5,23] Although advanced PS was a borderline significant independent poor factor on survival for indolent ATL in the model that used all patients, the factor was not a prognostic factor anymore when data were limited for only untreated patients (models C and D in Table 3). Among 12 patients who received chemotherapy, 7 (58%) had advanced PS at diagnosis. This suggests that patients with advanced PS at diagnosis might have a condition that required treatments, which introduced the disappearance of the effect of advanced PS on survival, even though advanced PS was an independent poor factor.

Regarding the effect of the presence of extranodal lesions on poor survival, we previously reported that BM involvement was a prognostic factor for aggressive ATL.[24] Although we did not present the effect of each extranodal lesion on survival in detail, we also confirmed that the survival rate of patients with BM involvement was significantly poor compared with patients those without BM involvement ($P = .04$; data not shown), but the survival rate of patients with skin involvement was not different compared with those without ($P = .66$; supplemental Figure 2). However, some studies reported that the presence of skin lesions was a possible poor prognostic factor in indolent ATL,[6,20-22] as described earlier. Setoyama et al[21] reported that smoldering cases with a deeper infiltration pattern had a more aggressive course than cases with a superficial infiltration pattern. Degree of skin involvement might be associated with prognosis in indolent ATL.

Previously, our study group noted that some patients showed alterations in tumor suppressor genes (p16 INKA[25,26] or p53[27]) or aneuploidy greater than 1 chromosomal locus by comparative genomic hybridization in ATL cells[28] and that such abnormalities were associated with a poor prognosis. Although we could not perform molecular analyses for all patients in the present study, 7 were examined molecularly, and at least one abnormality was found in each patient (data not shown). They had a poor prognosis and died within 2.5 years. Patients with a poor prognosis who died during the first steep slope in the survival curve (Figure 1A) might have had such genetic alterations.

The primary purpose of this study was to analyze prognosis of smoldering and chronic types together as an indolent type of ATL. Therefore, we were not able to present in detail the difference in

prognostic factors between subtypes, which is one of the limitations in this study. The number of cases evaluated in this study was too small to perform detail analyses for prognostic factors in indolent ATL. Further large-scaled studies are warranted.

In conclusion, the long-term prognosis of patients with indolent ATL was not good without a plateau phase in the survival curve. Further studies are warranted to elucidate patients with indolent ATL who require intensive chemotherapy, allogenic hematopoietic stem cell transplantation (in cases of aggressive ATL), or combination therapy with zidovudine and interferon alfa.[29,30] In addition, new molecular targeting treatments, such as histone deacetylase inhibitors,[31] which have shown promise in the treatment of $CD4^+$ cutaneous T-cell lymphoma, should be taken into consideration for treatment of indolent ATL.

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Authorship

Contribution: Y.T. collected and analyzed the data and wrote the manuscript; M.I. analyzed the data and wrote the manuscript; Y.I., M.T., T.J., T.K., Y.Y., S.K., S.I., Y.M., and M.T. made the diagnoses and treated the patients with ATL; and K.T. organized the study.

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CORRESPONDENCE

Is Zidovudine and Interferon-Alfa the Gold Standard for Adult T-Cell Leukemia-Lymphoma?

TO THE EDITOR: Bazarbachi et al[1] recently reported the results of a meta-analysis on the use of zidovudine (AZT) and interferon-alfa (IFN) in adult T-cell leukemia-lymphoma (ATL). They performed a retrospective survey of 254 patients treated at several institutes in which AZT/IFN has been routinely used for the treatment of ATL, and compared the overall survival (OS) between patients who received first-line AZT/IFN and those who received conventional chemotherapy. On the basis of the obtained data, they concluded that AZT/IFN should be considered the gold standard of first-line therapy for leukemic ATL because of better OS in patients with acute, chronic, and smoldering ATL treated by first-line AZT/IFN than chemotherapy.

This treatment approach has not been extensively evaluated in Japan, a major endemic area for ATL, mainly because of the lack of approval of both agents for the treatment of ATL under national health insurance. Therefore, the promising results involving a large number of patients encourage us to perform prospective clinical trials in Japan.

Several points should be taken into consideration when interpreting the Bazarbachi et al[1] data. Most important, the characteristics of patients treated with the two first-line treatment modalities, AZT/IFN and conventional chemotherapy, appear to be similar; however, the decision process to select the therapeutic modality for each patient should be described in more detail to make relevant comparisons. Second, the reasons a fraction of patients treated with conventional chemotherapy subsequently received maintenance AZT/IFN should be described. In addition, the possibility of interference with OS by second-line chemotherapy following AZT/IFN, and by allogeneic hematopoietic stem-cell transplantation, which is considered one of the recommended options for younger patients[2] following either AZT/IFN or chemotherapy, should be discussed.

We reported the results of a multi-institutional phase II study, Japan Clinical Oncology Group (JCOG) 9303, of VCAP (vincristine, cyclophosphamide, doxorubicin, and prednisone), AMP (doxorubicin, ranimustine, and prednisone), and VECP (vindesine, etoposide, carboplatin, and prednisone), in which the median survival time (MST) in 56 patients with acute ATL was 10.9 months.[3] The results were similar in the subsequent phase III study, JCOG 9801; MST and 3-year OS were 12.7 months and 23%, respectively (unpublished data). On the other hand, the MST in patients with acute ATL treated with AZT/IFN and chemotherapy was 9 and 6 months, respectively.[1] It should be noted that the MST achieved by chemotherapy and by AZT/IFN reported by Bazarbachi et al appears worse than in the JCOG chemotherapy studies, although there is a possible bias of patients presenting with a more favorable condition in the phase III study, partly because of the eligibility criteria for prospective clinical trials.

On the other hand, the Bazarbachi et al[1] results with AZT/IFN in patients with smoldering and chronic (ie, indolent) ATL are promising in view of potentially establishing a new effective ATL treatment. We recently reported a 5-year OS of as low as 47.2% in patients with indolent ATL who were mainly observed by a watchful waiting policy until disease progression.[4] Surprisingly, Bazarbachi et al[1] reported 100% OS beyond 5 years; however, the number of patients with indolent ATL in their study (n = 17) was too small to conclude that AZT/IFN is the standard of care in this cohort. Furthermore, the reasons some patients received first-line chemotherapy instead of AZT/IFN or watchful waiting should be described more precisely, and a comparison with the OS in patients who had been observed without intervention until progression should be included.

Considering the promising but preliminary nature of the Bazarbachi et al[1] findings, we are now planning a randomized phase III study that compares the outcome of AZT/IFN versus watchful waiting in patients with indolent ATL in Japan. This study will seek to establish the standard of care for patients with indolent ATL in the near future.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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ORIGINAL ARTICLE: CLINICAL

Pretreatment total serum protein is a significant prognostic factor for the outcome of patients with peripheral T/natural killer-cell lymphomas

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Abstract

Peripheral T- and NK-cell lymphomas (PT/NKCLs) are relatively rare, and few studies have validated the International Prognostic Index (IPI) for PT/NKCLs in prospective clinical trials. Histopathological specimens from 136 patients, enrolled in six prospective multicenter trials of doxorubicin-containing regimens, with PT/NKCLs were reviewed by six hematopathologists following the WHO classification. This combined analysis demonstrated that the IPI was not predictive of prognosis for patients with PT/NKCLs as previously shown by GELA. In a univariate analysis, low total serum protein (TP) and albumin levels, gastrointestinal tract involvement, and histologic subtype (extranodal NK/T-cell lymphoma, nasal type, and peripheral T-cell lymphoma, unspecified) were significantly associated with reduced survival. In a multivariate analysis, TP ($p = 0.004$) and histologic subtype ($p = 0.024$) remained significant. We discuss the need to establish the importance and meaning of TP and to develop new strategies for patients with PT/NKCLs allowing for TP, especially with worse histologic subtypes.

Keywords: *International Prognostic Index, peripheral T-cell lymphoma, total protein, WHO classification*

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Introduction

Peripheral T-cell lymphomas (PTCLs) are relatively rare neoplasms and not equally distributed geographically throughout the world. For instance, PTCLs account for 10% of all non-Hodgkin lymphomas in Western countries [1], but 23% in Japan [2]. Advances in immunophenotyping and molecular genetics have defined several distinct entities of PTCLs that were not identified by the Working Formulation (WF) classification [3]. The third edition of the World Health Organization (WHO) classification system [4] specifies 16 major subtypes of T- and natural killer (NK)-cell lymphomas with characteristic morphologic and immunophenotypic features and clinical manifestations. Although this classification allows different subgroups of patients with T-cell lymphoma to be identified, it does not inform on patient outcome.

The International Prognostic Index (IPI) [5], which was originally constructed for all aggressive lymphomas and is the most widely used index worldwide, has also been reported to predict the prognosis of patients with PTCLs [6–8]. However, the IPI has not been validated for patients with PTCLs who are enrolled in multicenter prospective clinical trials incorporating central pathology review by several pathologists using the WHO classification [4]. Among adult aggressive lymphomas, the T-cell phenotype is associated with a poor prognosis [9–12], except for anaplastic large cell lymphoma (ALCL) [1,8,12]. However, only a few studies have examined prognostic factors or models for PTCLs. A study conducted by the Intergruppo Italiano Linfomi demonstrated that the Prognosis Index for peripheral T-cell lymphoma, unspecified (PTCL-U) (PIT) can be used to predict the survival of PTCL-U [13].

A retrospective analysis of the prognosis of peripheral T- and NK-cell lymphomas (PT/NKCLs) based on retrospective clinical reviews is somewhat cumbersome, primarily due to the heterogeneity of treatment regimens. For this reason, we undertook a combined analysis of multicenter prospective trials conducted by the Japan Clinical Oncology Group – Lymphoma Study Group (JCOG–LSG) during the 1990s with the main endpoint of developing a practical and reproducible method to predict the precise prognosis for patients with PT/NKCLs, as did the Groupe d'Etude des Lymphomes de l'Adulte (GELA), in which patients were treated following the LNH87 or LNH93 protocol [14].

Materials and methods

Patient selection

This study, JCOG0108A, analyzed 1141 patients enrolled in the following six JCOG–LSG multicenter clinical trials for advanced adult aggressive lymphomas according to the WF classification [3] in those days, which were conducted consecutively in the 1990s: JCOG9002, 9203, 9505, 9506, 9508, and 9809. Patients with mycosis fungoides, Sézary syndrome, adult T-cell leukemia/lymphoma (ATLL), and precursor T-lymphoblastic leukemia/lymphoma (T-ALL/LBL) were excluded from all of the studies. Detailed descriptions of the patient eligibility criteria for these six trials were previously described [15–19]. All of the protocols described above, including the informed consent document, were approved by both the JCOG Protocol Review Committee and the institutional review board of each institution. The first edition of JCOG0108A was written on 27 June 2001 and approved by the JCOG Protocol Review Committee on 31 October 2001.

Treatment

All patients were enrolled in multicenter prospective studies and treated with doxorubicin (DXR)-containing second- or third-generation multidrug combination chemotherapies [15,16], or cyclophosphamide (CPA), DXR, vincristine (VCR), and prednisolone (PSL) (CHOP)-like regimens [17–19]. JCOG9002, 9203, and 9809 were clinical trials for patients with all IPI [15,16,19], while JCOG9505 and 9506 were those for high-intermediate/high-risk [17,18], and JCOG9508 was a trial for low-intermediate/low-risk of IPI [18].

Histopathological and immunohistochemical analyses by central review

An expert panel of six hematopathologists (Kiyoshi Mukai, Shigeo Nakamura, Koichi Ohshima, Masahiro Kikuchi, Yoshihiro Matsuno, and Tadashi Yoshino) and two clinicians (Tomomitsu Hotta and Masanori Shimoyama) reviewed the histopathologic diagnosis for 1023 of the 1141 patients enrolled in the six studies. The panel was provided with essential clinical data, including the age and gender of each patient, biopsy site, anatomic disease distribution, and anti-human T-cell leukemia virus type 1 antibody status. A consensus diagnosis was reached following histological review of each biopsy specimen in accordance with the third edition of the WHO classification system [4], including 136 patients diagnosed with PT/NKCLs. Immunohistochemistry using formalin-fixed paraffin-embedded sections and a panel of antibodies (see 'Appendix. Supporting Information') was used. T/NK-cell lineage was assigned only when the neoplastic lymphoid cells expressed at least one of the T/NK-cell antigens, such as CD3, CD45RO,

or CD56, and did not stain for the examined B-cell antigens (CD20 or CD79a).

ALCL was diagnosed for cases with a typical anaplastic morphology or sinus involvement, and a non-T/non-B or a T-cell phenotype, which was identified only by immunohistochemistry, and strong, uniform CD30 expression. For pathological diagnosis of extranodal NK/T-cell lymphoma, nasal type (NKTCL), patient samples were examined by Epstein–Barr virus-encoded small RNA-1 *in situ* hybridization (EBER-ISH). Southern blotting for the T-cell receptor gene rearrangement was not performed.

Prognostic factors

Eighteen clinical, biochemical, and radiologic parameters were analyzed to evaluate their capacity to predict patient outcome. These prognostic factors and risk groups, as defined by the IPI [5], were subjected to univariate analysis. Then, significant variables were included in a Cox multivariate analysis.

Except for the following incomplete data sets, complete data sets were available for all 136 patients with PT/NKCLs, which were diagnosed by central pathological review using data on all prognostic variables as well as overall survival (OS): seven patients did not have accurate pretreatment serum lactate dehydrogenase (LDH) levels, mainly because of a lack of information on the upper limit of the normal range for each institution at that time; one patient was not accurately staged and evaluable for performance status (PS); three patients were not evaluable precisely for the presence of B symptoms; and five, six, four, and four patients did not have pretreatment total serum protein (TP), albumin (Alb), and aspartate transaminase (AST) levels, and hemoglobin (Hb) levels determined, respectively.

Statistical analysis

Multiple statistical analyses were performed by a statistician (Kenichi Yoshimura) in the JCOG Data Center. Comparisons between patient groups were analyzed using a χ^2 test for categorical variables. The OS was calculated using the date of enrollment in each study until the date of death or last follow-up for living patients, and OS curves were estimated using the Kaplan–Meier method. The log-rank test was used to assess the significance of unadjusted differences in OS for each prognostic factor. Prognostic factors with $p < 0.3$ by univariate analysis were included in a multivariate analysis. The multivariate analysis was performed by the Cox proportional hazards model to identify subsets of independent prognostic factors for OS. Two-sided t tests were used to calculate all p-values and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SAS, version 9.1.3 (SAS Institute, Inc., Cary, NC).

Results

Major clinical characteristics and prognosis of PT/NKCLs compared to diffuse large B-cell lymphoma

The major clinical and biologic characteristics of PT/NKCLs and diffuse large B-cell lymphoma (DLBCL) are summarized in Table I. The statistically significant characteristics for PT/NKCLs were advanced stage, two or more extranodal sites, poorer PS, and presence of B symptoms. When 127 patients with PT/NKCLs with complete data sets available for the IPI were grouped by risk factors, PT/NKCLs showed a higher grade than those with DLBCL (Table I).

The 5-year OS of patients with PT/NKCLs was 46% (95% confidence interval [CI], 38–54%), which was significantly inferior to the 58% 5-year OS (95% CI, 54–62%) of patients with DLBCL. The IPI separated the patients with DLBCL into four risk groups with distinct survival outcomes (data not shown). On the other hand, the OS of patients with PT/NKCLs in the high-risk group was not less than that of patients in either the high-intermediate- or low-intermediate-risk groups (Figure 1), indicating that the IPI model does not fit well for patients with PT/NKCLs.

Table I. Comparison of clinical and biologic characteristics between patients with diffuse large B-cell lymphoma and peripheral T- and NK-cell lymphomas.

Parameter	DLBCL ($n=642$) (%)	T- and NK-cell lymphomas ($n=136$) (%)	p-Value
Sex (male/female)	60/40	67/33	0.13
Age (≤60/>60)	59/41	70/30	0.02
Ann Arbor stage (I+II/III+IV)	38/62	19/81	<0.001
Extranodal sites (≤1/≥2)	79/21	70/30	0.03
ECOG PS (0+1/≥2)	84/16	70/30	<0.001
LDH (≤N/>N)	47/53	41/59	0.24
B symptoms (no/yes)	75/25	47/53	<0.001
IPI (L/LI/HI/H)	38/31/20/11	27/33/22/18	0.04

DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; N, normal; IPI, International Prognostic Index; L, low risk; LI, low-intermediate risk; HI, high-intermediate risk; H, high risk.