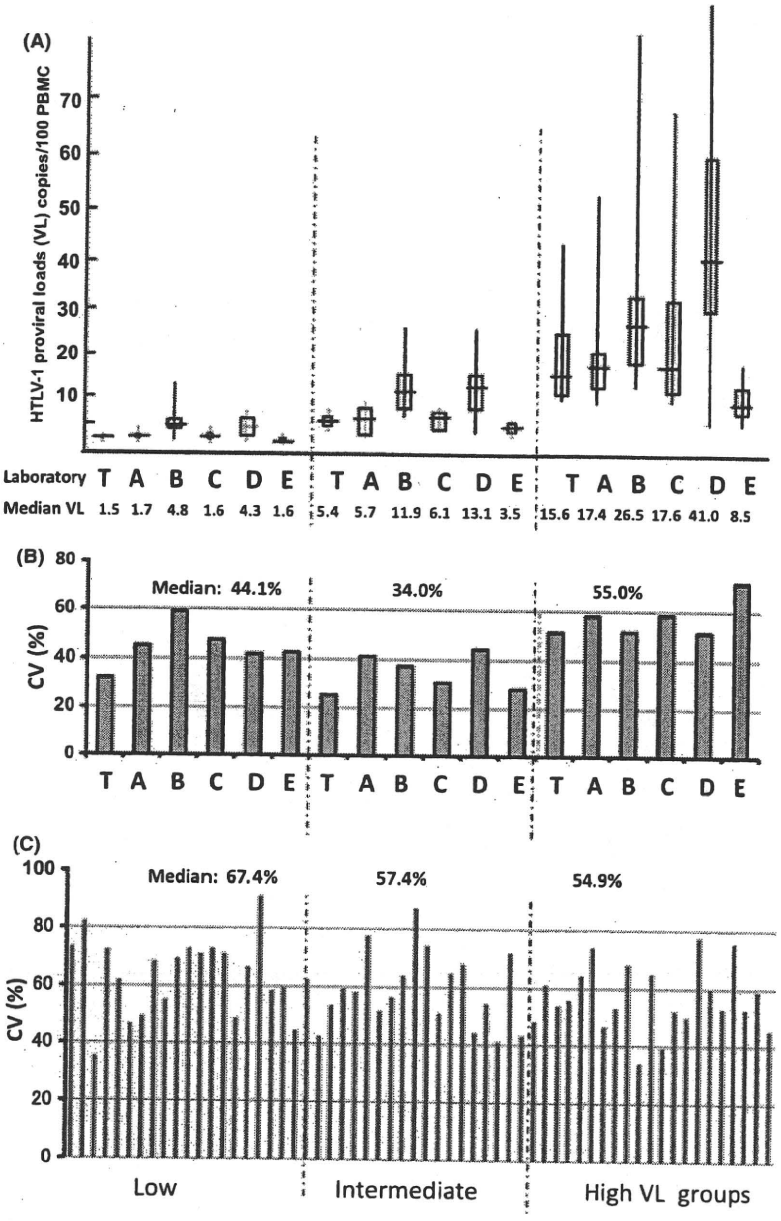

Fig. 1. Variation in human T-cell leukemia virus type-1 (HTLV-1) proviral loads (VL) quantification at the three VL levels in the first study. The three VL levels were divided based on the VL measured at a core laboratory T. Ranges of VL values at each level were as follows: low level (0–2.1, $n = 20$); intermediate level (3.0–7.7, $n = 20$); and high level (10.2–43.2, $n = 20$). (A) Intra-laboratory variability of measured VL at each VL level. The box plots show the median (horizontal line), interquartile range (box) and range (whiskers) in each laboratory (T, A, B, C, D and E). The *y*-axis shows measured VL copy numbers per 100 peripheral blood mononuclear cells (PBMC). (B) Intra-laboratory coefficient of variations (CV) (%) at three VL levels. The CV values were calculated based on the VL measured in each laboratory. (C) Inter-laboratory CV (%) for individual samples. Each CV value was calculated based on the measured VL in six laboratories.

ratory CV for 60 samples was 59.9% (range, 34.2–93.4%), consisting of 67.4% (range, 35.7–82.3%) in the low level group, 57.4% (range, 41.2–87.4%) in the intermediate group and 54.9% (range, 34.2–77.6%) in the high level group, respectively. Although inter-laboratory CV (%) was very wide by sample, there was a good correlation in the scatter plots of each VL between values measured in the core laboratory T and those measured in the other five laboratories. The fitted linear regression curves are shown in Figure 2. The inter-laboratory correlation coefficients ranged from 0.760 to 0.875, indicating that VL were measured with good precision in each laboratory. However, inter-laboratory regression slopes differed among laboratories. The slopes of laboratories A and C were close to 1.0 (0.992 and 0.984, respectively), indicating that the measured VL in the two laboratory systems were similar to values that were measured in the core laboratory T. However, the slopes of laboratory B and D were greater than 1.0 (1.393 and 2.206, respectively), indicating that the VL measured were always higher than in the core laboratory T. In contrast, the slope of laboratory E was <1.0 (0.399), indicating that the VL were always lower than in the core laboratory T. However, it is well recognized that the difference in slope influences all samples equally when a good correlation is observed as a systematic deviation.[12] Therefore, in this paper we derived an original "adjustment coefficient" in order to standardize data by calculating an inverse value of the slope (1/slope) for each laboratory. The "adjustment coefficient" for each laboratory ranged from 0.453 to 2.51 (Table 2).

Inter-laboratory variability after sharing RM in the second study. We considered that the large variation among assay systems in the first study might be related to some factor affecting universal measurements of samples, such as the RM and normalization, which are essential for qPCR. Therefore, we conducted the second study.

In the second study, the median VL of 20 samples in five laboratories (T, A, B, C and D) were 4.9, 6.6, 2.7, 4.5 and 3.4

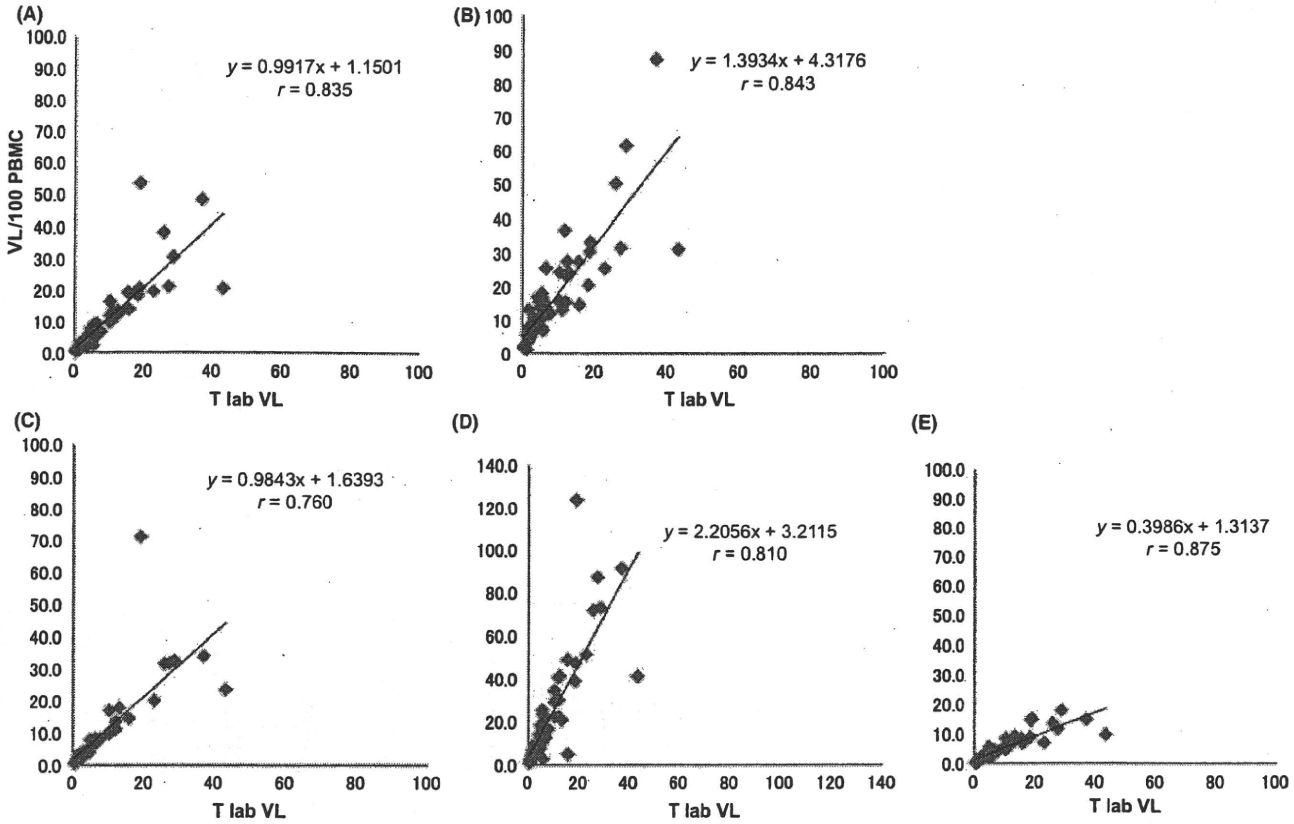

Fig. 2. Inter-laboratory comparison of human T-cell leukemia virus type-1 (HTLV-1) proviral loads (VL) in linear regression analysis. Scatter plot of VL of 60 samples between the core laboratory T and the other five laboratories (A), (B), (C), (D) and (E). PBMC, peripheral blood mononuclear cells.

copies per 100 PBMC, respectively. The maximal difference in the median VL was 2.4-fold (A *vs* B). The median inter-laboratory CV for VL, raw pX copy number and IC gene copy number before normalization (the first study) and after normalization (the second study) are shown in Figure 3A–C, respectively. The median inter-laboratory CV for VL was slightly reduced (from 59.9% to 48.2%) with no statistical significance. The median CV for raw pX copy number was reduced significantly from 66.9% to 35.3% ($P < 0.05$), whereas those for the VL and IC gene copy number remained statistically unchanged. We also performed linear regression analyses in a similar way to the first study. Data from the first and second studies are summarized in Table 3. This discrepancy of inter-laboratory CV between the raw copy number and VL appeared to account for poor IC accuracy and precision. Therefore, we next examined the effect of the IC gene copy assays for inter-laboratory CV of VL.

Table 2. Summary of inter-laboratory variability of VL in the first study and an original adjustment coefficient for standardization

Index of inter-laboratory variability	Laboratory				
	A	B	C	D	E
Correlation coefficient†	0.835	0.843	0.760	0.810	0.875
Slope†	0.992	1.393	0.984	2.206	0.399
Adjustment coefficient (=1/slope)	1.008	0.718	1.016	0.453	2.506

†These indexes were derived from linear regression analyses for 60 measured VL between each of the five laboratories (A, B, C, D, E) and the core laboratory T set as a reference (=1.0).

Quality of IC gene copy assays. To confirm whether a large variation in IC gene copy measurements is involved in the lack of improvement of standardization of VL in each assay system, we evaluated the measurement quality of the IC gene copy assay in each laboratory. The "IC accuracy" was defined as the measured copy number relative to the expected copy number with an input genomic DNA dose of 50 ng. This amount corresponds to approximately 16 600 copies based on one copy per 3 ng of genomic DNA. The "IC precision" was evaluated by using the median CV (%) of 60 measurements by qPCR with the respective IC gene. The results are summarized in Table 4, showing that both "IC accuracy" and "IC precision" were superior in RNase P, β-globin and CD81 compared with β-actin.

Discussion

Many studies have reported that a VL is linked to the pathogenesis of a virus. The HTLV-1 VL is thought to be equivalent to the HTLV-1-infected cell number.[13,14] However, in contrast to RT-qPCR for a large amount of transcripts, it is difficult to accurately discriminate a small concentration of the HTLV-1 provirus at a level of 10^{-2} to $^{-3}$.[12] The reliability of in-house assay systems has been previously evaluated as the quality of reproducibility, but little information is known about the intra- and inter-laboratory variations in HTLV-1 VL measured by different in-house qPCR systems that are set up independently at each laboratory. It is desirable that all assay systems can

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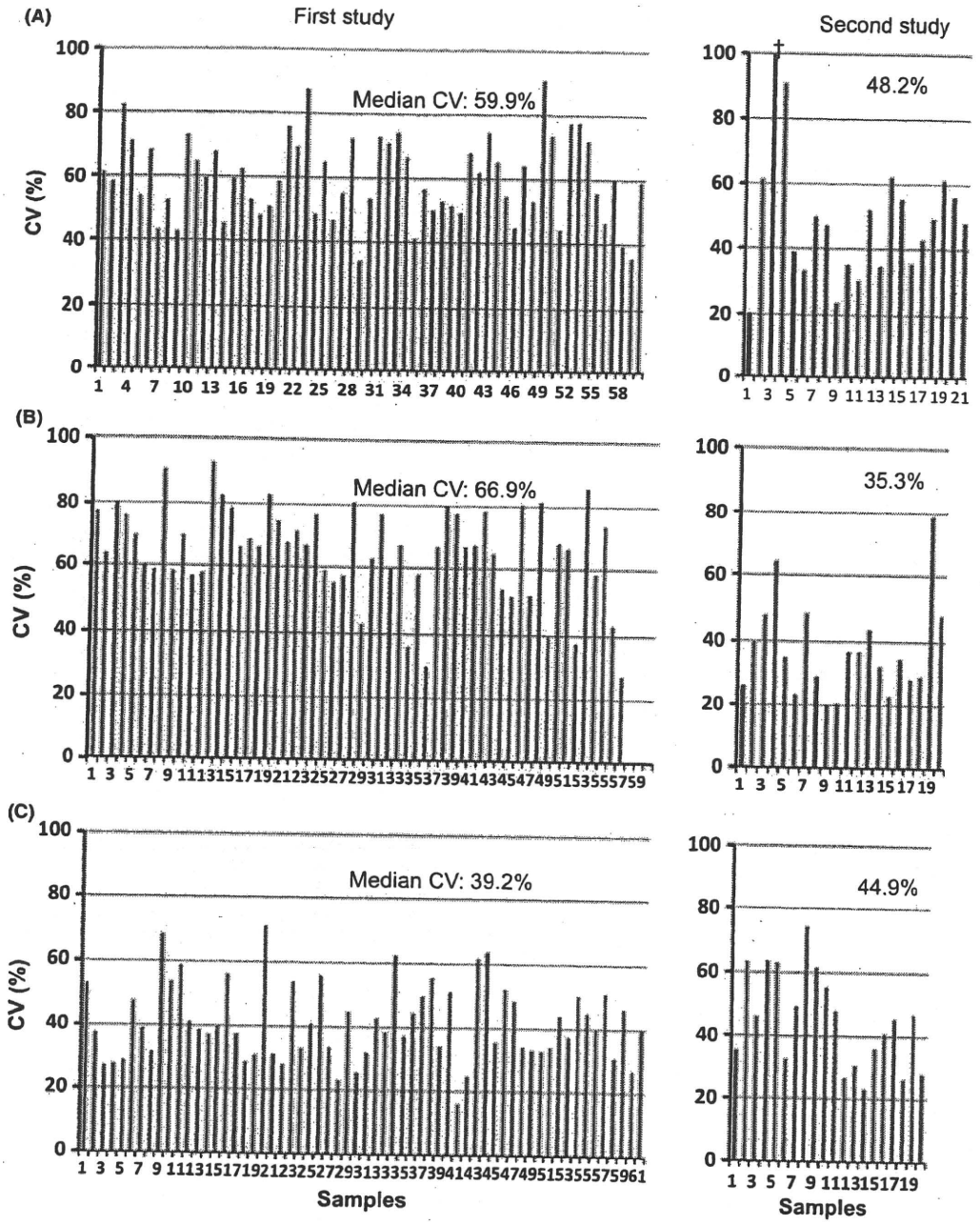

Fig. 3. Comparison of inter-laboratory coefficient of variations (CV) in individual samples for (A) proviral loads (VL), (B) raw pX copy number before normalization and (C) IC gene copy in the first and second studies. The median CV for only the raw pX copy number significantly decreased compared with that of the first study (66.9 versus 35.3%, $P < 0.05$). †The data was scaled over (144.7%).

measure VL accurately anytime and anywhere,[15,16] but to our knowledge there is no information on this point.

In this paper, we discovered three interesting findings in the first study. First, an extremely wider variation than we expected was seen in the measured values for HTLV-1 VL, especially in the group of high VL samples, from much lower copies to 100 copies or more per 100 PBMC. This is probably explained by the biological characteristics of the HTLV-1 virus, such as defects or mutations in target regions of primers and probes, or multiple integration of the proviral genome.[17] Second, we found a large difference in the actually measured VL between laboratories with a maximal difference of 3.7-fold in the median value. Third, the inter-laboratory correlation coefficients were excellent between the core laboratory T and each of the other laboratories. The second and third findings together indicate that each in-house assay system works well individually, but there was a systematic deviation from the expected values due to the difference in assay systems (Table 1). Such systematic deviation can be easily adjusted with an additional factor like our original "adjustment coefficient (an inverse value of the slope)" (Table 2) to compensate the data. However, such compensation alone is not enough to standardize the HTLV-1 VL measurement and to explain the wide intra- and inter-laboratory variability. Therefore, we performed the second study.

Table 3. Summary of inter-laboratory variability of VL in the first and second studies

	First study	Second study	*P*-value
CV%, median (range)			
VL	59.9 (34.2–93.3)	48.2 (20.0–144.3)	
Raw pX copy/50 ng DNA dose	66.9 (30.1–91.6)	35.7 (20.5–79.0)	<0.05
IC gene copy/50 ng DNA dose	39.2 (16.2–71.2)	44.9 (23.0–74.0)	
Index of linear regression analysis†			
VL			
Correlation coefficient	0.824	0.815	
Slope	1.195	1.05	
pX raw copy/50 ng DNA dose			
Correlation coefficient	0.761	0.811	
Slope	1.513	1.055	
PCR efficiency			
pX	1.976	1.981	
IC	1.967	1.98	

†Indexes were derived from linear regression analyses for measured VL between each of the five laboratories and the core laboratory T set as a reference (=1.0).

Table 4. Accuracy and precision for internal control (IC) gene copy assays in each laboratory

	IC gene used in laboratory†					
	T (core)	C	B	D	A	E
	RNP	RNP	β-actin	β-actin	β-globin	CD81
IC accuracy‡						
Measured/Expected	1.345	0.869	2.522	1.816	1.051	1.041
IC precision§						
CV (%)	11.8	13.2	35.1	32.6	11.4	13.8

†Rnase-P(RNP), β-globin and CD81 were expected to be at a tolerable level, but not β-actin. ‡"IC accuracy" was defined as the measured copy number relative to the expected copy number with an input genomic DNA dose of 50 ng. §"IC precision" was evaluated by using median coefficient of variation (CV) (%) of 60 measurements by quantifiable polymerase chain reaction with the respective IC gene.

In the second study, we standardized the RM for pX using plasmid provirus DNA for each in-house assay system, based on our inference that the main cause of the universal difference in the VL by laboratory might be due to the difference in RM. The inference was confirmed as presented in Table 3, where the inter-laboratory CV for raw pX copy number was reduced significantly after the RM standardization. Moreover, we found that IC gene measurements were also important for standardization of VL measurements as presented in Table 4, showing that both "IC accuracy" and "IC precision" were superior in RNase P, β-globin and CD81 compared with β-actin. Nevertheless, the most relevant IC gene for HTLV-1 provirus quantification remains unclear.

In general, quantitative variations by qPCR are affected by many factors, such as biological variations, process variations, systemic variations and other biased variations. Now a key question arises: which grade of intra- or inter-laboratory CV is acceptable for measurement of HTLV-1 VL? Of the in-house qPCR for HTLV-1 VL assays, most studies have described only a test performance by evaluating intra- and inter-assay variability using the same sample. For example, a report by Nagai[18] states that acceptable intra- and inter-assay CV values are around 25%. On the other hand, there are few studies on intra- and inter-laboratory variability indispensable for HTLV-1VL standardization. We searched the literature for qPCR assays in herpes viruses that were similar to our study. The studies reported that the median CV values of intra- and inter-laboratory variations about 20 and 40% for Cytomegalovirus (CMV)[19], and about 40 and 135 for Epstein-Barr virus (EBV)[20], respectively. Interestingly, intra-laboratory CV were <40% and significantly smaller than inter-laboratory CV. Although their study designs were not always the same as ours, the grade of median intra-laboratory CV values for HTLV-1, 44.9% (range, 25.4–71.8%), in our study was a little larger than that for CMV and EBV, whereas the grade of median inter-laboratory CV value for HTLV-1 in our study, 59.9% (range, 34.2–93.4%), was smaller than that for the two viruses. However, the grades could not be simply compared between HTLV-1 and other viruses because the viruses were biologically different to each other and the VL assay systems were also different.

Similarly, the grade of intra-laboratory CV for genomic genes have been reported to be 25–30% based on the quality of reproducibility within a single in-house assay.[5,9] Compared with the grade of the previous data, our intra-laboratory CV of 44.9% (range, 25.4–71.8%) seemed to be greater. This may come from the difference in the study design and analyzing procedures; previous studies focused on evaluating the quality of reproducibility of one sample in a single assay, whereas the present study focused on evaluating how much variability is present in the measurement of the same 20 samples in three VL levels in each laboratory, as described by Addona *et al.*[11] Therefore, the most relevant grade of intra-laboratory CV for HTLV-1 provirus quantification remains unclear.

Regarding a grade of inter-laboratory variation for measurement of HTLV-1 VL, we were unable to compare this with other studies because the present study is the first to investigate the variation. We have shown that the grade of median inter-laboratory CV% was 59.9% (range, 34.2–93.4%), and the value decreased as the measured value increased from low to high (Fig. 1C), which presumably reflects the effects of stochastic phenomena operative at a low input template copy number. So far, most healthy carriers have a low VL of less than three copies per 100 PBMC,[21] and it is considered that a VL of 5–10 copies per 100 PBMC is the critical level to be at risk of adult T-cell leukemia.[22] Therefore, we need a measure of high accuracy and precision for real-time qPCR of proviral DNA equivalent to the actual number of infected cells. Taken together, it is desirable that both grades of intra- and inter-laboratory CV for HTLV-1 VL measurement is <20%. The present study indicates that the 20% of inter-laboratory CV for measurement of VL is feasible, because the inter-laboratory CV for the pX copy number reduced by one-second (66.9% to 35.7%). Unfortunately, although it was not achievable in this series, as summarized in Table 4, poor IC accuracy and precision were responsible for insufficient improvement of inter-laboratory CV for VL. Accordingly, if high accuracy and precision of IC, such as RNP, beta-globin and CD81 is selected, 20% of inter-laboratory CV for VL seems to be realistically feasible.

In conclusion, in this study, we have shown that there is a variety in real-time qPCR assay systems, and a wide variability in intra- and inter-laboratory values for quantifying HTLV-1 VL. To improve the accuracy and precision of the quantification, standardization of HTLV-1 VL using appropriate RM (plasmid DNA) and relevant IC genes is necessary.

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Disclosure Statement

The authors have no conflict of interest.

Abbreviations

CV	coefficient of variation
HTLV-1	human T-cell leukemia virus type-1
IC	internal control
PCR	polymerase chain reaction
qPCR	quantifiable polymerase chain reaction
RM	reference material
VL	proviral load

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広範囲 血液・尿化学検査 免疫学的検査

—その数値をどう読むか—
［第7版］

(3)

IX. 免疫学的検査　I. 細胞性免疫

CD抗原によるグループ分類

T細胞系CD抗原，B細胞系CD抗原，骨髄細胞系CD抗原—造血器腫瘍細胞を中心に—

鶴田一人　濵﨑典子　松野貴子
森内拓治　跡上　直　上平　憲

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T細胞系CD抗原，B細胞系CD抗原，骨髄細胞系CD抗原—造血器腫瘍細胞を中心に—

Flow cytometric cell analysis by surface antigen according to CD grouping

鶴田一人　濵﨑典子　松野貴子
森内拓治　跡上　直　上平　憲

Key words：CD分類，CD抗原，造血器悪性腫瘍，FCM，WHO分類

1. 概　　説

血液細胞の表面膜ないし細胞内には，幹細胞から成熟細胞に至る分化レベルに相応した分化抗原が存在し機能している．この分化抗原は，発見の歴史や機能・構造の特性より接着分子，受容体，リガンド/サイトカイン，ケモカインなどとも呼ばれ，細胞を識別するマーカーとして使われている．これら抗原を特異的に認識するモノクローナル抗体群をCD分類[1-3]として整理統合されている(2007年9月現在　CD350分類)．ヒト造血器細胞抗原の解析方法は，免疫組織化学的染色法とフローサイトメトリー(FCM)法に大別される．FCM法は，多重染色法などで詳細・精確な抗原の同定や少数腫瘍細胞の検出にも応用される．正確なCD抗原の同定には目的とする細胞集団を適格に選択(gating)することが不可欠である．そのgatingの方法は，鏡検の形態学に相当する前方・側方散乱光から構成される光学的形態(FSC/SSCサイトグラム)によるものとCD45抗原(蛍光強度)と光学的側方散乱光を組み合わせたCD45-gating法が一般に使われている．そしてこのgatingされた中の標的細胞の抗原プロファイルより細胞の系統性・分化度・成熟度・亜型・サブセットなどの解析が行われる．現在，造血器腫瘍の分類はWHO分類[4,5]を中心に進められている．

したがって，本稿では末梢血や骨髄血で，しばしば遭遇する造血器腫瘍細胞のモノクローナル抗体(MoAb)の反応性，すなわち対応するCD抗原プロフィールについて，WHO分類に準じて概説する．

2. 検査の目的

(1) 造血器腫瘍細胞の表面膜・細胞内の(分化)抗原を調べ，T細胞系・B細胞系・骨髄細胞系の系統性・分化度などから細胞の特性を同定，ひいては病型診断する．

(2) T・B・NK細胞およびそのサブセットから細胞性免疫能の評価をする(他稿参照)．

3. 試料の採取方法，保存条件

a. 対象となる試料

ヘパリン加末梢血・骨髄血および胸・腹水，脳脊髄液，肺胞洗浄液などの体液．細切浮遊したリンパ節・脾臓・肝臓・腫瘍性腫瘤．

b. 保存条件

室温保存し，速やかに測定することが望ましい．翌日検査時は，細胞培養液にて4℃保存．末梢全血は室温保存する．長期の保存は適当な

Kazuto Tsuruda, Noriko Hamasaki, Takako Matsuno, Takuji Moriuchi, Sunao Atogami, Shimeru Kamihira: Department of Laboratory Medicine, Nagasaki University Hospital　長崎大学病院 検査部

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固定液で固定後保存(ただし，固定に不向きな抗原もある).

4. 測 定 法

一般臨床検査としては，全血(末梢血，骨髄血)を用いた直接ないし間接免疫蛍光抗体法で染色後FCMにて測定する.

(1) 末梢血(100 μL)・骨髄血(約1×10^6 cells/mL)に規定量の蛍光色素標識MoAbを加える.
(2) 室温(遮光)で15分反応.
(3) 溶血剤を2mL加えて約3分室温静置.
(4) 500gで5分間遠心後，上清の除去.
(5) PBS 0.5mLに浮遊させ，FCM測定.

5. 基 準 値

抗原陽性の判定は，使用抗体と同じアイソタイプの陰性コントロール抗体で染色したコントロールとの蛍光強度の差で決める．実際の症例では蛍光ヒストグラムパターンのみでは判定困難のものもあり，蛍光/散乱光サイトグラムを併用し，陰性・陽性を判断することが望ましい[6].

6. 生理的変動

正常細胞は年齢に応じて変動するものもある．腫瘍細胞は病勢，治療により大きく変動する.

7. 臨床的意義

CD抗原の同定は，腫瘍細胞の特性や同定に不可欠である．FCMではサイトグラムのパターンと抗原profileの2つの方法で腫瘍性細胞を判断する．次に腫瘍細胞であるならば系統性(T細胞系，B細胞系，骨髄細胞系など)，そして分化・成熟度，サブセットの抗原系の有無から腫瘍細胞の帰属を評価し，病型診断の主要な根拠となる．また，抗原profileの特性から染色体/遺伝子異常の予測や治療方針の決定に活用される.

ここでは検査のパラダイムに従い，まず系統性を決めるCD抗原，続いて分化度(特に未分化型)を評価するCD抗原，次に各T細胞系・B細胞系・骨髄細胞系腫瘍の病型ごとのCD抗原の発現特性について，WHO分類に準じて述べる.

a. 腫瘍細胞の系統性評価のCD抗原

腫瘍細胞をT細胞系，B細胞系，骨髄細胞系の3大系統別に分ける抗体は，cytoplasmic(c)/surface(s)CD3，TCRα/β・γ/δ，cCD79a，c/sCD22，cMPOである．T細胞系の系統性の決定は，特異性の観点からc/sCD3，TCRα/β・γ/δの抗原発現の検出をもって判断される．特にcCD3抗原の検出が最も重要で，pro-Tの時期から発現する．B細胞系はcCD79a，c/sCD22が用いられる．cCD79a抗原はpro-B段階において発現が認められ形質細胞(細胞内)に至るまで発現は継続しているが一部T細胞とも反応する．骨髄細胞系ではcMyeloperoxidase(cMPO)抗原の検出がhallmarkとなっている．最近では，特に未分化な白血病芽球が疑われる場合，cCD79a，cCD3，cMPOの3重染色を行い，一挙に系統性の決定が行われている.

b. 未分化・未熟型細胞の評価用CD抗原

使用抗体：CD34，CD117，cTdT，CD1a，CD99.

CD34抗原は，最も未分化な多能性造血幹細胞と全細胞系統の造血前駆細胞に発現する．CD117抗原(c-kit)は骨髄系幹細胞に発現する．cTerminal deoxynucleotidyl transferase(cTdT)抗原は，核内マーカーでT・Bリンパ球前駆細胞に発現している．CD1a抗原はTCR(−)の胸腺皮質T細胞に検出され，この時期CD4$^+$・CD8$^+$の同時発現も認められるが，これも未熟型T細胞として評価できる．CD99はTリンパ芽球性白血病を抗原に作成されたMIC2遺伝子の産物である糖蛋白に反応する.

c. T細胞系腫瘍の評価用CD抗原

1) 未熟型T細胞性腫瘍評価(表1)

使用抗体：cTdT，cCD3，CD1a，CD2，CD3，CD4，CD5，CD7，CD8，CD34，CD99.

Tリンパ芽球性白血病/リンパ芽球性リンパ腫(T-ALL/LBL)は，胸腺内で起こる分化によりpro-T，pre-T，cortical-T，medullary-Tの4つのステージに分類される．pro-T-ALLは

表1 未熟型T細胞性腫瘍(T-ALL/LBL)の免疫学的表現型

	cTdT	cCD3	CD7	CD2	CD1a	CD4	CD8	sCD3	CD34
Pro-T	+	+	+	−	−	−	−	−	+/−
Pre-T	+	+	+	+	−	−	−	−	+/−
Cortical-T	+	+	+	+	+	+	+	−	−
Medullary-T	+	+	+	+	−	+/−*	−/+*	+	−

cCD3: 細胞内CD3, sCD3: 細胞表面膜CD3.
*どちらか一方が陽性.

cTdT$^+$・cCD3$^+$・CD7$^+$陽性でCD34$^{+/-}$を示す. pre-T-ALLは続いてCD2$^+$が陽性となる. cortical-T-ALLでは, CD1a$^+$が陽性となり, またCD4$^+$・CD8$^+$の2重陽性が認められCD34$^-$は陰性化する. medullary-T-ALLでは, sCD3$^+$が陽性でCD4$^{+/-}$, CD8$^{-/+}$の発現は一定でない.

2) 成熟型T細胞性腫瘍評価(表2)

正常成熟T細胞には, CD2$^+$, CD3$^+$, CD5$^+$, CD7$^+$が発現しているが, 腫瘍細胞はこれらの一部に欠損が認められることが多い. 更に, CD4とCD8の発現により成熟型のCD4$^+$腫瘍とCD8$^+$腫瘍に大別され, CD30$^+$は未分化大細胞リンパ腫(ALCL)の診断に重要である.

a) 成熟型CD4$^+$腫瘍評価

使用抗体: CD2, CD3, CD4, CD5, CD7, CD25, HLA-DR.

主な成熟型CD4$^+$腫瘍細胞には, T細胞性前リンパ球白血病(T-PLL), 末梢T細胞リンパ腫, 分類不能型(PTCL, NOS), 血管免疫芽球性T細胞リンパ腫(AITL), 菌状息肉症/Sézary症候群(MF/SS), 成人T細胞白血病/リンパ腫(ATLL)がある. T-PLLは主病変が末梢血で前リンパ球が増殖するaggressiveなT細胞性白血病で, CD7$^+$陽性が診断的に重要である. AITLは, 全身性の末梢T細胞リンパ腫で, リンパ節へのpolymorphicな浸潤, 高内皮細静脈および濾胞樹状細胞の著明な増生を伴う多彩な細胞浸潤を特徴とする. 最近, CD10$^+$, CXCL13$^+$, PD-1$^+$が陽性であることが判明した. ATLLは, HTLV-1ウイルスにより引き起こされる腫瘍でCD3$^+$・CD4$^+$・CD25^{++}・CCR4$^+$・HLA-DR$^{+/-}$・CD7$^{-/+}$・CD26$^-$の腫瘍である. 予後・治療の観点から臨床病型分類(急性型, 慢性型, リンパ腫型, くすぶり型)が用いられ, 免疫学的表現型との関係では, 急性型のaggressiveな症例はHLA-DR$^-$陰性化, 慢性型ではCD7$^+$陽性化, リンパ腫型ではCD4$^+$・CD8$^+$の2重陽性を示す症例が多数認められる. MF/SSではCD25$^-$陰性でありATLLとの鑑別点となる.

b) 成熟型CD8$^+$腫瘍評価

使用抗体: CD2, CD3, CD5, CD7, CD8, CD16, CD56, CD57, TCRα/β, TCRγ/δ.

主な成熟型CD8$^+$腫瘍は, TIA1およびグランザイムBの発現を伴うことが多い. T細胞大型顆粒リンパ球性白血病(T-LGL)は, CD3$^+$・TCRα/β^+・CD8$^+$・CD57$^+$(80%)を示し, NK細胞のマーカーであるCD16$^{+/-}$, CD56$^{-/+}$, CD57$^{+/-}$の発現は一定でない. 肝脾T細胞リンパ腫(HSTL)と原発性皮膚$\gamma\delta$Tリンパ腫ではCD8の発現は一定しないが, TCRγ/δ^+は発現し, 診断的価値が高い.

d. B細胞系腫瘍の評価用CD抗原

1) 未熟型B細胞性腫瘍評価(表3)

使用抗体: cTdT, cCD79a, c/sCD22, cIgM, CD19, CD10, CD20.

Bリンパ芽球性白血病/リンパ芽球性リンパ腫(B-ALL/LBL)は免疫学的形質により3ステージに分けられる. 最も未熟な前駆細胞をpro-B-ALL(early precursor B-ALL)と呼びcTdT$^+$・cCD79a$^+$・cCD22$^+$・CD19$^+$が陽性となる. 中間期前駆prepre-B-ALL(common ALL)ではCD10$^+$が加えて陽性となる. 後期前駆pre-B-ALLではcIgM$^+$(μ鎖)が陽性となる. WHO分類に示される染色体/遺伝子と免疫学的表現型との関連性から述べると, 9;22転座B-ALLはCD13$^{+/-}$, CD33$^{+/-}$の骨髄性抗原が陽性となり,

表2　成熟型T細胞性腫瘍の主な疾患における免疫学的表現型

neoplasms	CD3	CD4	CD8	CD7	CD5	CD2	TIA1	GrB/Per	CD30	CD25	CD56	CD16	CD57	BCL6	CD10	EBV	EMA
T-PLL	+	+	+/−	+	+	+	−	−	−	−	−	−	−	−	−	−	−
T-LGL	+	−	+	−/+	−/+	+	+	+	−	−	−	+	+	−	−	−	−
ATLL	+	+	−	−	+	+	−	−	−/+	++	−	−	−	−	−	−	−
Agg NK	+c	−	−/+	−	−	+	+	+	−	−	+	−	−	−	−	+	−
ENK/T, Nasal type	+c	−	−/+	−	−	+	+	+	−	−	+	−	−	−	−	+	−
EATL	+	−	−/+	+	−	+	+	+	−/+	−/+	−/+[#]	−	−	−	−	−	−/+
HSTL	+[*%]	−	+/−	+	−	+	+	−	−	−	+	−	−	−	−	−	−
SPTCL	+	−	+	+	−/+	+	+	+	−	−	+	−	−	−	−	−	−
MF/SS	+	+	−/+	−/+	+/−	+	−	−	−	−	−	−	−	−	−	−	−
primary cutaneous γδT-cell lymphoma	+[*]	−	−/+	−/+	−	+	+	+	−	−	+	−	−	−	−	−	−
primary cutaneous CD30+LPD	+	+	−	−	+/−	+	+	−/+	+	+	−	−	−	−	−	−	+/−
AITL	+	+	−	+	+	+	−	−	−	−	−	−	−	+/−	+/−	−[**]	−
PTCL, NOS[##]	+	+/−	−/+	−/+	−/+	+	−	−	−/+	−	−	−	−^	−^	−^	−	−
ALCL, ALK+	−/+	+/−	−/+	−/+	+/−	+/−	+	+	++	++	+/−	−	−	+	−	−	++
ALCL, ALK−	+/−	+/−	−/+	−/+	+/−	+/−	+/−	+/−	++	++	+/−	−	−	−	−	−	+

c: cytoplasmic CD3のみ陽性, [*]γδ型T細胞受容体, [%]一部の例はαβ型T細胞受容体を発現, [#]CD56はmonomorphic type EATLまたはType IIの一部に陽性, [**]EBVは腫瘍細胞には検出されないが, 背景のB細胞の一部に大部分例で検出される, [##]PTCL, NOSは単一の疾患単位ではなく, 複数の疾患の複合体のため免疫学的表現型も多様である, ^PTCL, NOSの一部はfollicular helper T-cell(TFH)に由来し, 時にCD57, CD10, BCL6を発現する.

T-PLL: T-cell prolymphocytic leukaemia, T-LGL: T-cell large granular lymphocytic leukaemia, ATLL: adult T-cell leukaemia/lymphoma, Agg NK: aggressive NI-cell leukaemia, ENK/T, Nasal type: extranodal NK/T-cell lymphoma, nasal-type, EATL: enteropathy-associated T-cell lymphoma, HSTL: hepatosplenic T-cell lymphoma, SPTCL: subcutaneous panniculitis-like T-cell lymphoma, MF/SS: mycosis fungoides and Sézary syndrome, primary cutaneous CD30+LPD: primary cutaneous $CD30^+$ T-cell lymphoproliferative disease, including primary cutaneous anaplastic T-cell lymphoma, AITL: angioimmunoblastic T-cell lymphoma, PTCL, NOS: peripheral T-cell lymphomas, not otherwise specified, ALCL: anaplastic large cell lymphoma, GrB: granzyme B, Per: perforin.

(Jaffe ES, et al: Introduction and overview of the classification of the lymphoid neoplasms. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed, p163, IARC Press, Lyon, 2008より引用)

表3 未熟型B細胞性腫瘍(B-ALL/LBL)の免疫学的表現型

	cTdT	cCD79a	CD19	CD10	CD20	cIgM(μ)	sIg
Pro-B	+	+	+	−	−	−	−
Prepre-B	+	+	+	+	−/+	−	−
Pre-B	+	+	+	+	+	+	−

cIgM: 細胞内IgM(μ鎖)

更に，CD66c$^+$，CD25$^+$の発現が認められる．11q23異常ALLでは，特にt(4；11)はCD19$^+$・CD10$^-$でpro-Bを示し，CD15$^+$も陽性を示す．染色体数50本以上の高2倍体性B-ALLはCD45$^-$陰性となる．1；19転座B-ALLはcIgM$^+$(μ鎖)陽性のpre-B-ALLを示す．

2) 成熟型B細胞性腫瘍評価(表4)

使用抗体：表面膜グロブリン：sIg，細胞内グロブリン：cIg，CD5，CD10，CD23，CD103，CD38，CD138．

成熟型B細胞の特徴はsIgの発現を認めることである．更に，腫瘍性の評価をsIg軽鎖であるκ鎖，λ鎖の間に20％以上の陽性率の差を認めるとき，clonalityありと判断される．CD5$^+$陽性を示す腫瘍には，慢性リンパ性白血病/小リンパ球性リンパ腫(CLL/SLL)とマントル細胞リンパ腫(MCL)があり，CD23の発現とsIg抗原量(蛍光強度中央値)から鑑別される．B-CLLでは免疫グロブリン抗原量が少ない(sIg$^+$; weak)ことが特徴となる．CD10$^+$陽性を示す腫瘍には，濾胞性リンパ腫(FL)，バーキットリンパ腫(BL)，B細胞性大細胞型リンパ腫(DLBCL)の一部がある．ほかの免疫形質特徴を示す疾患では，有毛細胞白血病(HCL)は，CD103$^+$，CD11c$^+$，CD25$^+$などが陽性となる．形質細胞性骨髄腫(PC)では，CD38^{++}・CD138$^+$・cIg$^+$陽性で，約7割の症例にCD56$^+$が認められる．このCD38^{++}の特徴からCD38^{++} gatingにより形質細胞を選択して解析がなされる．

e. 骨髄細胞系腫瘍評価のCD抗原(図1)

1) 顆粒球系細胞評価

使用抗体：cMPO，CD11b，CD13，CD15，CD16，CD33，CD35，CD65，CD117，HLA-DR．

正常骨髄細胞の分化と免疫学的表現型を**図1**に示した．骨髄系細胞のスタンダードマーカーはcMPO$^+$，CD13$^+$，CD33$^+$である．顆粒球系の白血病acute myeloblastic leukemia(AML)の分化度はCD34$^+$，CD117$^+$の陽性率が参考となりFAB分類のAML: M0，M1で高値を示す．AML: M3は，前骨髄球の腫瘍であるためHLA-DR$^-$が陰性を示すことが大きな特徴となる．前骨髄球以降の分化抗原発現としては，CD15$^+$，CD65$^+$は前骨髄球以降の分化をした細胞に発現し，CD11b$^+$は骨髄球以降の細胞に，CD16$^+$，CD35$^+$は後骨髄球以降の細胞に発現し，成熟とともにより強く発現する．

2) 単球系細胞評価

使用抗体：CD4，CD11b，CD14，CD64．

単球系の評価はCD4$^+$，CD64$^+$，CD11b$^+$，CD14$^+$を中心に判断される．CD14は最も知られた単球マーカーである．しかし，成熟型単球には強く反応するが，単芽球は陰性で前単球より陽性となるためAML: M4，M5aでは陰性を示す．CD11b$^+$，CD64$^+$は単球系細胞に強い陽性を示す．CD13$^+$とCD33$^+$の発現では，単球系細胞はCD33$^+$が強く反応する．

3) 赤芽球・赤血球系細胞の評価

使用抗体：CD235a，CD71，CD36．

CD235a$^+$(グリコホリンA)は前赤芽球から赤血球に至る分化段階に広く反応する．CD71$^+$(トランスフェリンレセプター)は，赤芽球のほかに活性化細胞にも反応する．CD36$^+$は血小板に最も多く発現し，赤芽球，単球，マクロファージ，血管内皮細胞にも認められAML: M6bで高発現している．

4) 巨核芽球・血小板系細胞の評価

使用抗体：CD41，CD61，CD42．

表4　成熟型B細胞性腫瘍の主な疾患における免疫学的表現型

neoplasm	sIg；cIg	CD5	CD10	CD23	CD43	CD103	BCL6	IRF4/MUM1	cyclinD1	ANXA1
CLL/SLL	+；−/+	+	−	+	+	−	−	(+PC)	−	−
LPL	+/−；+	−	−	−	−/+	−	−	+*	−	−
splenic MZL	+；−/+	−	−	−	−	−	−	−	−	−
HCL	+；−	−	−	−	−	+	−	−	+/−	+
plasma cell myeloma(PC)	−；+	−	−/+	−	−/+	−	−	+	−/+	−
MALT lymphoma	+；+/−	−	−	−/+	−/+	−	−	+*	−	−
follicular lymphoma(FL)	+；−	−	+/−	−/+	−	−	+	−/+#	−	−
MCL	+；−	+	−	−	+	−	−	−	+	−
diffuse large B-cell lymphoma(DLBCL)	+/−；−/+	−***	−/+##	NA	−/+	NA	+/−##	+/−**	−	−
Burkitt lymphoma(BL)	+；−	−	+	−	+/−	NA	+	−/+	−	−

+：90％を超える症例で陽性，+/−：50％を超える症例で陽性，−/+：50％未満の症例で陽性，−：10％未満の症例で陽性．

IRF4/MUM1：interferon regulating factor 4/multiple myeloma-1，ANXA1：annexin A1，PC：proliferation centres.

* plasma cell component positive，# grades 3a and 3bの一部，## germinal centre B-cell(GCB)のDLBCLはCD10とBCL6を発現する，** activated B-cell(ABC) typeのDLBCLは典型例でIRF4/MUM1陽性である，*** DLBCLの一部はCD5陽性である．

NA：not applicable，LPL：lymphoplasmacytic lymphoma，MZL：marginal zone lymphoma，MCL：mantle cell lymphoma.

(Jaffe ES，et al：Introduction and overview of the classification of the lymphoid neoplasms. In：WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues，4th ed，p160，IARC Press，Lyon，2008より引用)

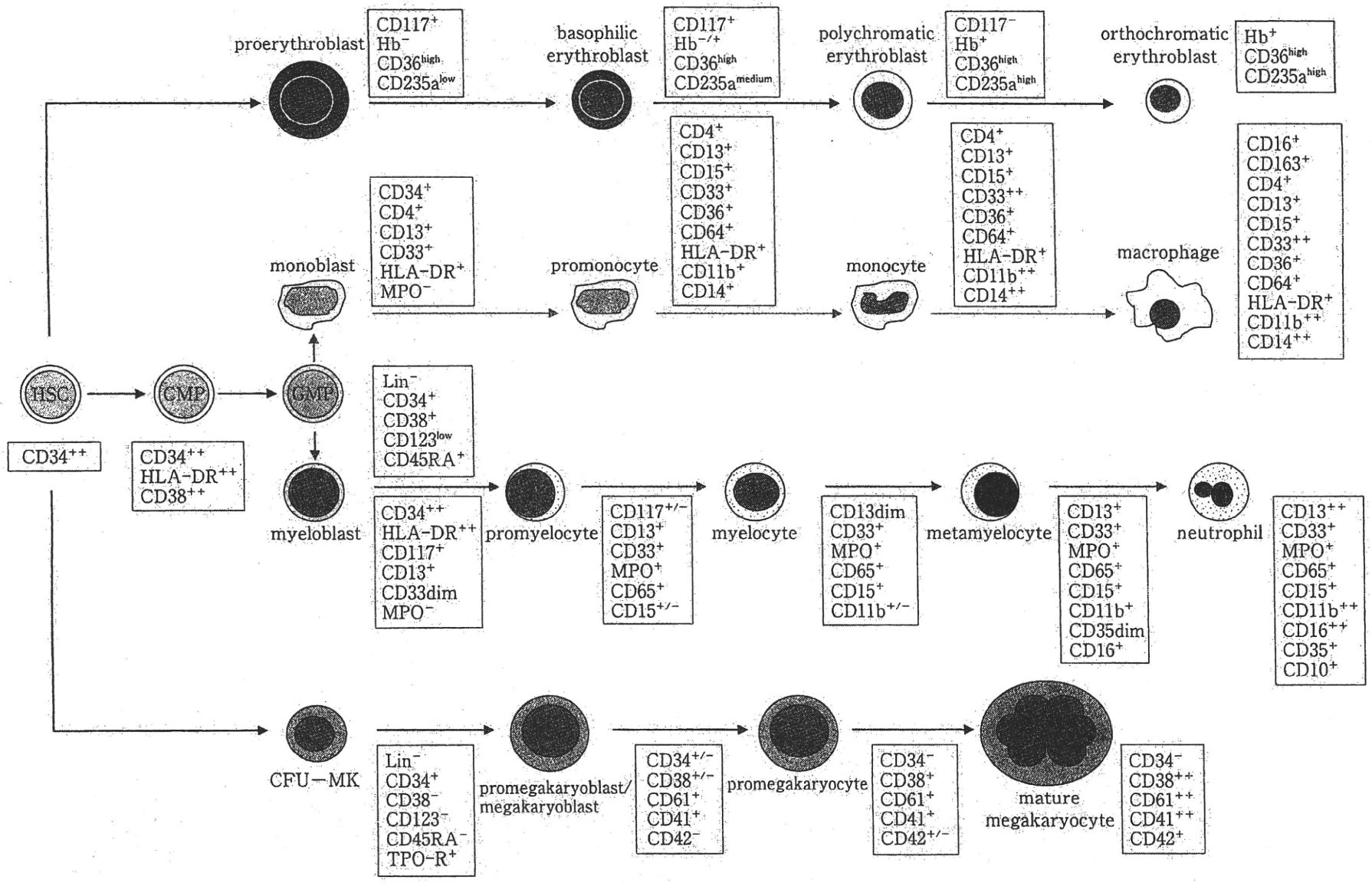

図1　正常骨髄細胞の分化と免疫学的表現型

(Jaffe ES, et al: Introduction and overview of the classification of the lymphoid neoplasms. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed, p22, IARC Press, Lyon, 2008 より引用)

巨核芽球細胞の検出にはCD41^{+}（グリコプロテインIIb），CD61^{+}（グリコプロテインIIIa）が用いられ検出率も高い．ほかにCD42（グリコプロテインIb）も用いられる．

8. 関連検査項目

MoAbによる造血器悪性腫瘍細胞検査，T細胞・B細胞百分率検査，T細胞サブセット検査，表面免疫グロブリン測定，TdT精密検査，顆粒球スクリーニング検査，FCMのtwo-color分析法による赤血球検査，リンパ球幼若化検査などがある．

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Trends in HTLV-1 Prevalence and Incidence of Adult T-Cell Leukemia/Lymphoma in Nagasaki, Japan

Yoshito Koga,[1] Masako Iwanaga,[2] Midori Soda,[3] Naoko Inokuchi,[1] Daisuke Sasaki,[1] Hiroo Hasegawa,[1] Katsunori Yanagihara,[1] Kazunari Yamaguchi,[4] Shimeru Kamihira,[1] and Yasuaki Yamada[1]*

[1]*Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[2]*Department of Hematology and Molecular Medicine, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[3]*Department of Epidemiologic Pathology, Radiation Effects Research Foundation, Nagasaki, Japan*
[4]*Department of Safety Research on Blood and Biological Products, National Institute of Infectious Diseases, Tokyo, Japan*

Most previous studies aimed at estimating the number of human T-cell leukemia virus type-1 (HTLV-1) carriers in endemic areas have been based on seroprevalence rates in blood donors; however, this may result in underestimation because of the healthy donor effect. People who have health problem do not donate blood. In the present study, the number of HTLV-1 carriers in Nagasaki City was estimated based on the seroprevalence rates in a hospital-based population from Nagasaki University Hospital. In accordance with previous reports, seroprevalence of HTLV-1 was higher in females, and year of birth-specific seroprevalence showed a significant annual decline in both genders (P for trend: <0.0001). The estimated number of HTLV-1 carriers in Nagasaki City was 36,983. The incidence of adult T-cell leukemia/lymphoma (ATLL) among HTLV-1 carriers was estimated using data from the Nagasaki Prefectural Cancer Registry. The estimated annual incidence of ATLL was 61 per 100,000 HTLV-1 carriers, and the crude lifetime risk of the development was 7.29% for males and 3.78% for females. There is a large pool of HTLV-1 carriers aged over 70 years, and a continuing development of cases of ATLL among the elderly is therefore expected. ***J. Med. Virol. 82:668–674, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: HTLV-1; ATLL; seroprevalence; retrovirus; epidemiology

INTRODUCTION

Human T-cell leukemia virus type-1 (HTLV-1) is the causative agent of adult T-cell leukemia/lymphoma (ATLL), HTLV-1-associated myelopathy/tropical spastic paraparesis, and HTLV-1-associated uveitis [Uchiyama et al., 1977; Poiesz et al., 1980; Yoshida et al., 1984; Gessain et al., 1985; Osame et al., 1987; Mochizuki et al., 1992]. HTLV-1 carriers are clustered in the southwestern districts of Japan, including Nagasaki, as well as among native Andeans, North Iranians, Central Africans, and African descendants in the Caribbean and South America [Tajima and Takezaki, 2003]. Such regional clustering is, at least in part, explained by vertical transmission of the virus by breast-feeding in closed communities [Kinoshita et al., 1984; Hino et al., 1985]. To prevent vertical transmission, the Adult T-Cell Leukemia/Lymphoma Prevention Program, which is a prefecture-wide breast-feeding intervention study for HTLV-1 carrier mothers, was initiated in Nagasaki in the middle of 1987 [Hino et al., 1996], and HTLV-1-positive pregnant women were advised to refrain from breast-feeding. It has also been demonstrated that HTLV-1 can be transmitted by blood transfusion, and routine screening of blood donors for anti-HTLV-1 antibodies has been conducted at all blood centers in Japan since 1986 [Inaba et al., 1999].

Previous studies found that age-specific rates of HTLV-1 seropositivity among residents and blood donors in endemic areas of Japan have declined annually [Tokudome et al., 1989; Yamaguchi et al.,

Dr. Koga and Dr. Iwanaga contributed equally to this work.

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*Correspondence to: Yasuaki Yamada, MD, PhD, Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. E-mail: y-yamada@nagasaki-u.ac.jp

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1992]. However, the results for HTLV-1 seropositivity in blood donors might have been underestimated, because blood is solely procured by voluntary donation in Japan and is limited to those aged 16–69 years old; in addition, people who have been notified that they are HTLV-1 carriers will not revisit a blood center. Furthermore, any age-specific analysis usually involves a birth cohort effect. Because the majority of Japanese HTLV-1 carriers were infected through mother-to-infant transmission, a birth cohort-specific analysis, rather than an age-specific analysis provides a more appropriate means of understanding trends in HTLV-1 seroprevalence.

Nearly 20 years have passed since the commencement of the Adult T-Cell Leukemia/Lymphoma Prevention Program and screening for anti-HTLV-1 antibodies in blood centers. The first aim of this study was to determine a more reliable estimate of the longitudinal trend in seroprevalence of HTLV-1 in Nagasaki City, using data from a hospital-based population from Nagasaki University Hospital. The second aim was to estimate the incidence of ATLL among HTLV-1 carriers by applying the hospital-based seroprevalence rate to ATLL records held in a population-based local cancer registry, namely, the Nagasaki Prefectural Cancer Registry [Soda and Ikeda, 1997].

MATERIALS AND METHODS

Patients

The results of anti-HTLV-1 antibody tests performed on 12,848 blood samples from 10,261 patients (males: 5,523; females: 4,738) who visited Nagasaki University Hospital between April 2000 and March 2007 were assessed retrospectively. Only the result of the first examination for each patient was considered, to avoid duplication of patients. Some patients were examined repeatedly at different times, but no patients produced conflicting results. The median age of the patients was 61 years (range: 0–99). Patients with HTLV-1-associated diseases were more likely to visit certain departments, such as the hematology, dermatology, neurology, and ophthalmology departments, and the data were therefore also analyzed by the department. The cardiovascular surgery department tested routinely for anti-HTLV-1 antibodies in all patients before surgery.

Anti-HTLV-1 Antibody Assay

The presence of anti-HTLV-1 antibodies in serum samples was assayed using a chemiluminescent enzyme immunoassay (CLEIA) (Lumipulse HTLV-1, Fujirebio, Inc., Tokyo, Japan) [Nishizono et al., 1991; Yoshiki et al., 1993], using an index cut-off value of 1.0. This assay is known to be less affected by non-specific reactions than the particle agglutination test [Fujiyama et al., 1995a,b; manufacturer's data sheet]. The validity of this assay was also verified in the Central Laboratory of Nagasaki University Hospital by comparison with the particle agglutination test (Serodia HTLV-1, Fujirebio, Inc.) and Western blotting (Genelabs Diagnostics Pte., Tsukuba, Japan). The sensitivity of CLEIA is adequate to detect almost all the HTLV-1 carriers, but there may be a small number of false-positive results (<0.5%) in which the HTLV-1 infection is not confirmed by the WHO criteria for Western blotting [unpublished observation; Fujiyama et al., 1995b]. However, the impact of this on the results of the current study was supposed to be marginal.

Statistical Analysis

The patients were categorized by year of birth into nine groups: before 1926, 1927–1936, 1937–1946, 1947–1956, 1957–1966, 1967–1976, 1977–1986, 1987–1996, and 1997–2007. The seroprevalence rates of HTLV-1 were calculated by dividing the number of seropositive subjects by the number of subjects tested in each group. The exact binomial 95% confidence intervals (CI) for seroprevalence were calculated as required. Comparisons of seroprevalence among categories were performed by calculating seroprevalence ratios and the 95% CI via a log-binomial regression model using PROC GENMOD. Trend tests were performed using the Cochran–Armitage test. Continuous data were compared using the Mann–Whitney test or the Kruskal–Wallis test. Post hoc analyses were performed using Dunn's multiple comparison test. Categorical data were compared using the χ^2 test or Fisher's exact test.

To estimate the year of birth-specific incidence of ATLL in HTLV-1 carriers, a two-step estimation procedure was performed. In the first step, the number of HTLV-1 carriers in the population of Nagasaki City was estimated by applying the sex- and year of birth-specific HTLV-1 seroprevalences of patients in Nagasaki University Hospital (excluding those in the departments usually involved in treating HTLV-1-related diseases, such as hematology, dermatology, neurology, and ophthalmology), to the 2006 census data for Nagasaki City (population size 454,203 in 2006). In the second step, the year of birth-specific incidence of ATLL in HTLV-1 carriers was estimated, based on the cases registered with the Nagasaki Prefectural Cancer Registry between 1990 and 2005 as the numerator, and the number of HTLV-1 carriers estimated in the first step as the denominator. The diagnosis of ATLL in the Nagasaki Prefectural Cancer Registry data was reevaluated by one of the authors (Y.Y.). The annual incidence of ATLL was estimated as the number of cases per 100,000 HTLV-1 carriers. The rates were compared using Poisson regression analysis, and relative risk (RR) and the 95% CI were calculated.

All statistical analyses were performed using the SAS version 9.1 (SAS Institute Japan, Tokyo, Japan) with a two-tailed significance level of 0.05. This study was approved by the institution's ethical committee and by the Nagasaki Prefectural Cancer Registry committee.

RESULTS

Seroprevalence of HTLV-1 in Patients

The presence of anti-HTLV-1 antibodies was confirmed in 1,392 (males: 653; females: 739) out of 10,261

TABLE I. HTLV-1 Seroprevalence and Age at Examination in Patients by Medical Department

Medical department	No. examined	Median age at examination (range)	No. positive for HTLV-1	Seroprevalence (%)
Hematology	1,052	55 (12–95)	223	21.20
Neurology	436	62 (12–99)	75	17.20
Dermatology	934	59 (0–92)	159	17.02
Ophthalmology	339	54 (5–86)	43	12.68
Cardiovascular surgery	1,417	69 (10–94)	180	12.70
Others	6,083	60 (0–97)	712	11.70
Total	10,261	61 (0–99)	1,392	13.57

patients. The overall seroprevalence was 13.57% (95% CI: 12.90–14.23%). Table I shows seroprevalence of HTLV-1 analyzed by department. As expected, the seroprevalence was higher among patients in the departments usually involved in treating HTLV-1-related diseases, such as hematology (21.2%), neurology (17.2%), and dermatology (17%). The seroprevalence among patients in the ophthalmology department was relatively low (12.68%) compared with those of the other three departments. The seroprevalence among patients attending the cardiovascular surgery department (12.7%) was comparable to that of the ophthalmology department and was slightly higher than that of the other departments not usually involved in treating HTLV-1-related diseases (11.7%); however, the difference was not significant ($P = 0.33$).

The overall seroprevalence was significantly higher in females than in males (15.6% vs. 11.82%, $P < 0.0001$) (Table II). This gender difference in seroprevalence existed in every year of examination, with a female/male ratio of around 1.3 (95% CI: 1.2–1.5). There was a trend toward a decline in annual seroprevalence during the study period from 14.53% in 2000 to 12.78% in 2007 (Fig. 1), but it was not statistically significant (P for trend: 0.22).

Seroprevalence of HTLV-1 in Patients by Year of Birth

Year of birth-specific seroprevalence rates showed a significant annual decline from 15.85% in those born before 1926 to 0% in those born during 1987–2007 in males (P for trend: <0.0001) and from 22.32% to 0% in females (P for trend: <0.0001) (Table III and Fig. 2). The annual decline in seroprevalence was especially rapid for those born between 1947 and 1976. The seroprevalence was consistently higher in females than males in each cohort born before 1966, but the predominance in females became weaker in those born after 1967.

Estimation of the Number of HTLV-1 Carriers in the Population

The sex- and year of birth-specific numbers of individuals corresponding to each birth cohort in the hospital data were extracted from the 2006 census data for Nagasaki City. Table IV shows the breakdown and the estimated number of HTLV-1 carriers in Nagasaki City based on the HTLV-1 seroprevalence of patients at Nagasaki University Hospital by year of birth. The estimated number of HTLV-1 carriers in Nagasaki City was 36,983 (12,755 males and 24,228 females). More than 90% of the carriers were born before 1966 and were thus more than 43 years old in 2009.

Estimation of the Incidence of Adult T-Cell Leukemia/Lymphoma Among HTLV-1 Carriers in Nagasaki City

On the basis of the Nagasaki Prefectural Cancer Registry data, a total of 360 ATLL cases (males: 188;

TABLE II. HTLV-1 Seroprevalence in Patients by Year of Examination and Gender

	Total		Male		Female		
Year of examination	No. positive/no. examined	Seroprevalence (%)	No. positive/no. examined	Seroprevalence (%)	No. positive/no. examined	Seroprevalence (%)	F/M ratio[a] (95% CI)
2000	195/1,342	14.53	88/717	12.27	107/625	17.12	1.4 (1.1–1.8)
2001	171/1,288	13.28	85/711	11.95	86/577	14.90	1.2 (0.9–1.6)
2002	169/1,172	14.42	87/649	13.41	82/523	15.68	1.2 (0.9–1.5)
2003	171/1,243	13.76	91/690	13.19	80/553	14.47	1.1 (0.8–1.4)
2004	163/1,289	12.65	76/699	10.87	87/590	14.75	1.4 (1.0–1.8)
2005	176/1,261	13.96	75/664	11.30	101/597	16.92	1.5 (1.1–2.0)
2006	182/1,375	13.24	79/721	10.96	103/654	15.75	1.4 (1.1–1.9)
2007	165/1,291	12.78	72/672	10.71	93/619	15.02	1.4 (1.1–1.9)
Total	1,392/10,261	13.57	653/5,523	11.82	739/4,738	15.60	1.3 (1.2–1.5)

95% CI, 95% confidence intervals.
[a]F/M ratio indicates the female-to-male ratio for seroprevalence.

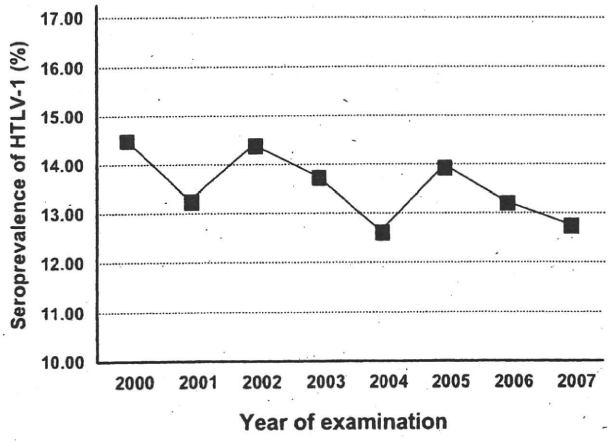

Fig. 1. Changes in annual seroprevalence of HTLV-1 in patients visiting Nagasaki University Hospital. There was an annual decline in seroprevalence, though this was not statistically significant (P for trend: 0.22).

females: 172) diagnosed between 1990 and 2005 (16 years) in Nagasaki City (Table V) were confirmed. The median age of the patients was 67 years (range: 33–99 years). No patient was born after 1967. The estimated annual incidence of ATLL was 61 per 100,000 HTLV-1 carriers, with a significantly higher rate in males (92) than females (44) (RR: 2.75, 95% CI: 2.37–3.19). There was a clear annual decline in prevalence by year of birth until 1966 (P for trend: <0.0001), with the highest rate in carriers born before 1926 (375 for males, 112 for females, and 171 in total) (Table V). The mean life span of Japanese individuals in 2008 was 79.19 years for males and 85.99 years for females, and the crude lifetime risk of developing ATLL in HTLV-1 carriers was thus 7.29% for males and 3.78% for females.

DISCUSSION

The results of this study indicate a clear annual decline in the year of birth-specific seroprevalence of HTLV-1, similar to that reported previously among blood donors in another area of Japan where the virus is endemic, namely, Kumamoto prefecture [Oguma et al., 1992; Yamaguchi et al., 1992]. The trend toward a rapid annual decline in HTLV-1 seroprevalence seen in the cohorts born from 1947 to 1976 is explained by improved sanitation, increased popularity of bottle-feeding, and the reduced duration of breast-feeding. The Adult T-Cell Leukemia/Lymphoma Prevention Program may have had a considerable impact on the decline in Nagasaki, because the seroprevalence in patients born after 1987, when this program was started, was zero. A decrease in seroprevalence of HTLV-1 has also been reported outside of Japan. In French Guiana, the prevalence in pregnant women older than 25 years was 8.3%, while that in those aged 25 years or younger was 2.8% [Tortevoye et al., 2000]. A recent article confirmed this trend [Tortevoye et al., 2005]. In a community-based follow-up study in Guinea-Bissau, the prevalence decreased from 3.5% in 1996 to 2.3% in 2006 [da Silva et al., 2009]. In the present study, a higher level of prevalence was found in females, which has been documented previously and was interpreted as showing that HTLV-1 is transmitted more easily from husband to wife than from wife to husband [Tajima et al., 1987; Kondo et al., 1989; Tokudome et al., 1989; Yamaguchi et al., 1992]. The recent reduction in this difference may be due to a decrease in the number of children and the frequent use of condoms as contraception in Japan.

Seroprevalence was higher in patients in the hematology, dermatology, and neurology departments than in other departments, because patients with HTLV-1-associated diseases visit these departments. In contrast, the prevalence in patients in the ophthalmology department was almost the same as that in patients in departments not usually involved in treating HTLV-1-related diseases. This is probably because of a low incidence of HTLV-1-associated uveitis. The prevalence in patients attending departments not usually involved in treating HTLV-1-related diseases was expected to indicate the background prevalence in Nagasaki City. The cardiovascular surgery department routinely tests

TABLE III. HTLV-1 Seroprevalence in Patients by Birth Year and Gender

	Total		Male		Female		
Year of birth	No. positive/no. examined	Seroprevalence (%)	No. positive/no. examined	Seroprevalence (%)	No. positive/no. examined	Seroprevalence (%)	F/M ratio[a] (95% CI)
Before 1926	246/1,316	18.69	117/738	15.85	129/578	22.32	1.4 (1.1–1.8)
1927–1936	458/2,568	17.83	224/1,465	15.29	234/1,103	21.21	1.4 (1.2–1.6)
1937–1946	312/1,961	15.91	139/1,079	12.88	173/882	19.61	1.5 (1.2–1.9)
1947–1956	236/1,710	13.80	115/915	12.57	121/795	15.22	1.2 (1.0–1.5)
1957–1966	91/990	9.19	36/481	7.48	55/509	10.81	1.4 (1.0–2.2)
1967–1976	37/910	4.07	18/456	3.95	19/454	4.19	1.1 (0.6–2.0)
1977–1986	12/588	2.04	4/281	1.42	8/307	2.61	1.8 (0.6–6.0)
1987–1996	0/155	0	0/78	0	0/77	0	—
1997–2007	0/63	0	0/30	0	0/33	0	—
Total	1,392/10,261	13.57	653/5,523	11.82	739/4,738	15.60	1.4 (1.2–1.5)[b]

95% CI, 95% confidence intervals.
[a]F/M ratio indicates the female-to-male ratio for seroprevalence.
[b]The F/M ratio was adjusted for the year of birth.

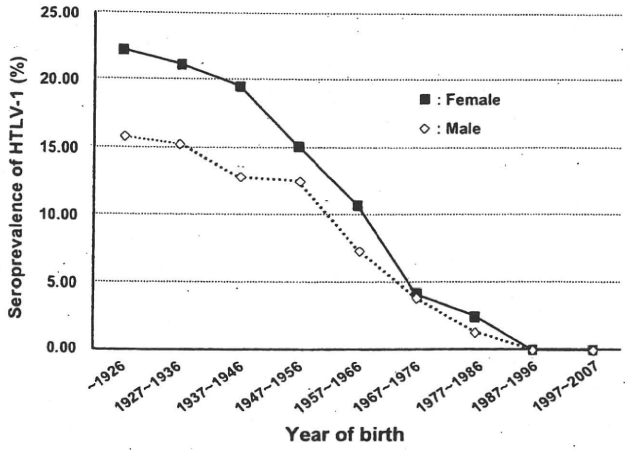

Fig. 2. Seroprevalence of HTLV-1 in patients by year of birth. The HTLV-1 seroprevalence rate showed a significant annual decline by birth year in both genders (P for trend: <0.0001).

for anti-HTLV-1 antibodies in all patients before surgery. The prevalence among patients in this department was 12.7%, which was slightly higher than that in other departments not usually involved in treating HTLV-1-related diseases (11.76%). This could be due to the fact that the median age of the patients in the cardiovascular surgery department (69 years) was greater than that in other departments (60 years).

Most previous studies that have examined seroprevalence of HTLV-1 among blood donors in endemic areas used age-specific but not year of birth-specific seroprevalence. The blood center of Nagasaki prefecture reported year of birth-specific seroprevalence based on data from 253,824 blood donors and found a 3.11% (7,889) seropositivity [Chiyoda et al., 2001]. The seroprevalence was 8.95% in donors born before 1936, 7.3% in those born between 1937 and 1946, 5.11% in those born between 1947 and 1956, 2.9% in those born between 1957 and 1966, 1.51% in those born between 1967 and 1976, and 1.29% in those born between 1977 and 1983. A recent report confirmed this trend [Iwanaga et al., 2009]. These prevalence rates are, however, approximately 50% lower than those estimated in the present study. The results based on blood donors do not reflect the real prevalence and are underestimates. However, there it is also possible that hospital-based prevalence results are overestimated. The seroprevalence of HTLV-1 was 0.3% in patients at a genitourinary medicine department at a hospital in southeast London, while those in blood donors in the north of England and north London were very low, in the range of 0.0014–0.005% [Turner et al., 2008]. The seroprevalence of HTLV-1/2 in pregnant women in Argentina (0.191%) was 10 times higher than that in blood donors (0.019%) [Trenchi et al., 2007].

The year of birth-specific annual incidence of ATLL among HTLV-1 carriers indicated a clear trend of higher rates in earlier year of birth, due to the tendency of this disease to develop in the elderly. The crude lifetime risk of developing ATLL in HTLV-1 carriers was 7.29% for males and 3.78% for females. A study in Saga prefecture, another area of Japan in which HTLV-1 is endemic, estimated age- and sex-specific prevalence of HTLV-1 among blood donors [Tokudome et al., 1989]. The sex- and age-specific numbers of HTLV-1 carriers were also estimated by applying the results to the entire population of Saga prefecture. The crude annual incidence rates among 100,000 HTLV-1 carriers of 40–79 years old were 115.9 for males and 66.4 for females, and the cumulative risks were 4.5% for males and 2.6% for females. Two other studies found similar results. The Ehime ATLL study group reported that the crude annual incidence rate among 100,000 male carriers aged over 30 years was 145.3 while that for females was 55.2 [Kondo et al., 1989]. In a recent cohort study of the populations of the small islands in Nagasaki prefecture, the crude annual incidence rate among 100,000 carriers of 30 years old or older was estimated at 137.7 for males and 57.4 for females, and the cumulative risk for individuals of 30–79 years of age was estimated at approximately 6.6% for males and 2.1% for females [Arisawa et al., 2000]. It is interesting that the lifetime

TABLE IV. Estimation of the Number of HTLV-1 Carriers in the Population

	Size of population[a]		Seroprevalence of HTLV-1 (%)[b]		Estimated no. HTLV-1 carriers[c]		
Year of birth	Male	Female	Male	Female	Male	Female	Total
Before 1926	9,107	19,993	12.98	20.38	1,182	4,075	5,257
1927–1936	19,916	28,268	13.41	19.18	2,671	5,422	8,093
1937–1946	24,519	29,823	10.67	18.56	2,616	5,535	8,151
1947–1956	36,358	38,642	9.16	12.30	3,330	4,753	8,083
1957–1966	26,808	29,887	6.34	9.15	1,700	2,735	4,434
1967–1976	27,368	29,991	3.69	3.90	1,010	1,170	2,180
1977–1986	23,628	26,050	1.04	2.07	246	539	785
1987–1996	23,341	22,809	0	0	0	0	0
1997–2007	19,175	18,520	0	0	0	0	0
Total	210,220	243,983	9.99	14.28	12,755	24,228	36,983

[a]Data were obtained from the 2006 census data for Nagasaki City.
[b]Data were based on the results of patients at Nagasaki University Hospital, with the exception of the departments of hematology, dermatology, neurology, and ophthalmology, during the years of examination (2000–2007).
[c]The rate was calculated by multiplying sex- and year of birth-specific numbers by sex- and year of birth-specific HTLV-1 seroprevalence.

TABLE V. Estimation of the Incidence of Adult T-Cell Leukemia/Lymphoma Among HTLV-1 Carriers in Nagasaki City

	Estimated no. HTLV-1 carriers[a]			No. adult T-cell leukemia/ lymphoma in NPCR[b]			Estimated annual incidence of adult T-cell leukemia/lymphoma among HTLV-1 carriers (per 100,000)[c]		
Year of birth	Male	Female	Total	Male	Female	Total	Male	Female	Total
Before 1926	1,182	4,075	5,257	71	73	144	375	112	171
1927–1936	2,671	5,422	8,093	60	52	112	140	60	86
1937–1946	2,616	5,535	8,151	30	24	54	72	27	41
1947–1956	3,330	4,753	8,083	24	18	42	45	24	32
1957–1966	1,700	2,735	4,434	3	5	8	11	11	11
1967–1976	1,010	1,170	2,180	0	0	0	0	0	0
1977–1986	246	539	785	0	0	0	0	0	0
1987–1996	0	0	0	0	0	0	0	0	0
1997–2007	0	0	0	0	0	0	0	0	0
Total	12,755	24,228	36,983	188	172	360	92	44	61

[a]Data are cited from Table IV.
[b]We used cases registered with the Nagasaki Prefecture Cancer Registry (NPCR) during 1990–2005 (a total of 16 years).
[c]The rate was calculated using number of adult T-cell leukemia/lymphoma in the NPCR as the numerator and the estimated number of HTLV-1 carriers as the denominator.

risk of developing ATLL in HTLV-1 carriers in the present study was higher than those reported in other studies, although it is possible that the number of HTLV-1 carriers was overestimated because of the hospital-based nature of the current study. In this context, it is necessary to consider the shift in age distribution of patients: the median age of patients before 1990 in Nagasaki prefecture was younger than 65, but that in recent years was over 70 (data from Nagasaki Prefectural Cancer Registry). If we therefore evaluate the lifetime risk for a group in which the patient age is limited to 79, the results will exclude patients older than this age. The number of HTLV-1 carriers is apparently decreasing in most areas of the world in which HTLV-1 is endemic, including Nagasaki. However, it is also true that the incidence of ATLL, at least in Nagasaki City or the Nagasaki prefecture, has not changed in the past 20 years (data from Nagasaki Prefectural Cancer Registry). There is still a large pool of elderly HTLV-1 carriers over 70 years old in Japan, and the continuous development of ATLL among individuals in this pool is expected. Further studies in HTLV-1 endemic areas outside Japan are needed to confirm these trends.

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