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Impact of cystatin C elevation and albuminuria on probability of adverse outcomes in HIV-infected men receiving HAART

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Key words
albuminuria – cancer – cardiovascular disease – cystatin C – HIV

Abstract. Background: Highly active antiretroviral therapy (HAART) has contributed to the longevity of human immunodeficiency virus (HIV)-infected patients; however, improved survival has been accompanied by an increase in the prevalence of kidney disease. Kidney disease may be partly responsible for higher morbidity in HIV-infected patients than in HIV-uninfected subjects. Methods: A total of 515 well-controlled HIV-infected men on HAART was enrolled in a 3-year prospective cohort study. The incidence of cancer and CVD was investigated over time. The impact of cystatin C elevation and albuminuria at baseline on the incidence of each disease was examined. Albuminuria was estimated by determining the albumin-to-creatinine ratio (ACR). The cumulative incidence of cancer and CVD was analyzed using the Kaplan-Meier method, stratified by the presence and absence of elevated cystatin C and albuminuria biomarkers. Cox proportional hazards analysis was used to calculate the hazards ratio (HR) and 95% incidence interval (CI) of each biomarker, adjusted for known risk factors. Results: All participants completed the 3-year follow-up study. During the follow-up period, cancers and CVD developed in 13 (2.5%) and 14 (2.7%) participants, respectively. The Kaplan-Meier estimates were significantly increased for cancer incidence in patients with cystatin C elevation and for CVD in those with albuminuria. The HR (95% CI) of cystatin C elevation for occurrence of cancer was 6.09 (1.30 – 24.6) and the HR (95% CI) of ACR $\geq$ 20 mg/g for CVD was 8.97 (2.20 – 60.8). Conclusions: Cystatin C elevation and/or albuminuria at baseline in HIV-infected men undergoing HAART may be associated with poor prognosis.

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Introduction

The introduction of highly active antiretroviral therapy (HAART) has markedly reduced acquired immunodeficiency syndrome (AIDS)-related deaths and the number of opportunistic infectious diseases, which were the main causes of mortality among human immunodeficiency virus (HIV)-infected subjects in the pre-HAART era [1, 2, 3]. However, the causes of mortality have shifted to non-AIDS-related diseases in the contemporary HAART era [4]. Currently, the major causes of death include cardiovascular disease (CVD) and non-AIDS-defining cancers, and these deaths are reported to occur at an earlier age in the HIV-infected population than in the general population [5, 6, 7]. Results of previous studies have suggested that HIV-infected participants are at risk for higher morbidity and mortality compared with HIV-uninfected participants, partly because there is often a high prevalence of several chronic comorbidities, including chronic kidney disease (CKD), among those infected with HIV [8, 9].

Inflammation is now recognized as an underlying condition which is relevant to incidence and persistence of a variety of chronic diseases. Elevation of C-reactive protein (CRP), interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α is commonly used as an index of systemic inflammation. Albuminuria and elevated serum cystatin C levels are originally used as markers for detecting early kidney disease, but now are regarded as new surrogate markers for systemic inflammation as well. Albuminuria reflects the presence of endothelial injury, probably caused by increased levels of proinflammatory cytokines due to kidney dysfunction [10, 11]. Cystatin C provides prognostic information beyond its role as an index of kidney dysfunction and may be a better overall measure of the spectrum of pathophysiologic abnormalities that

accompany kidney disease [12, 13, 14, 15]. Early identification of the presence of albuminuria or elevated cystatin C may guide clinicians to an earlier intervention, regardless of co-existence of kidney disease. Indeed, previous reports have suggested that cystatin C and albuminuria might be associated with CVD and all-cause mortality among patients currently infected with HIV [16, 17]. However, the cohorts in those studies were somewhat heterogeneous with regard to the frequency of HAART and the extent of HIV infection control.

Here, we conducted a 3-year prospective cohort study of well controlled HIV-infected men receiving HAART to identify the impact of cystatin C elevation and albuminuria on the incidence of cancer and CVD.

Materials and methods

Study design

This study was a prospective cohort study aiming to ascertain the relationship between baseline clinical characteristics and the new onset of cancer and CVD during a 3-year follow-up period. The adverse outcomes included incidence of cancer and CVD. CVD was defined as incidence of acute myocardial infarction, heart failure, cerebral infarction, and any other cardiovascular and cerebrovascular events. The study was performed in accordance with the Declaration of Helsinki and was approved by the institutional review board (approval certificate no. 681-09-1-13). Informed consent was obtained from all participants.

Study population

The study cohort consisted of 515 ambulatory HIV-infected men (mean age; 47.4 ± 11.3 years) with estimated glomerular filtration rates (eGFR) of more than 15 ml/min/1.73 m^2 at entry, who were recruited from routine outpatient HIV care at Tokyo Metropolitan Komagome Hospital. They were consecutively enrolled between February and April 2008. The participants were followed for at least 6 visits per year over a 3-year period. The mean CD4 cell count was 440 ± 207/μl, and all subjects received HAART and had undetectable HIV-RNA levels (< 50 copies/ml) at baseline. Tenofovir disoproxil fumarate (TDF), abacavir, and zidovudine were administered to 279 (54.2%), 98 (19.0%), and 91 (17.7%) participants, respectively. Atazanavir/ritonavir, lopinavir/ritonavir, fosamprenavir/ritonavir, and efavirenz were given to 195 (37.9%), 67 (13.0%), 8 (1.6%), and 218 (42.3%) participants, respectively.

Measurements

Nonfasting blood and spot urine samples were collected randomly for analysis as part of a routine clinical visit. Albuminuria was measured using a turbidimetric immunoassay to determine the urinary albumin-to-creatinine ratio (ACR), and was arbitrarily categorized into 4 ranges: normal range, < 10 mg/g; middle-normal range, 10 – 19 mg/g; high-normal range, 20 – 29 mg/g; and abnormal range, ≥ 30 mg/g. The ACR was determined by at least 2 consecutive assays conducted 3 months apart. Serum cystatin C was measured using the latex agglutination-turbidimetric immunoassay (IATRO Cys-C; Mitsubishi Chemical Medicine Corporation, Tokyo, Japan), with a cut-off value of 1.0 mg/l. EGFR was based on serum creatinine (Cr) and was calculated using the 3-variable Japanese equation developed by the Japanese Society of Nephrology: eGFR (ml/min/1.73m^2) = 194 × Serum $Cr^{-1.094}$ × $Age^{-0.287}$ × 0.739 [18]. CD4 cell counts in HIV-infected subjects were determined using a specific monoclonal antibody and fluorescence-activated cell-sorter analysis. HIV-RNA copy levels were determined using the Roche Amplicor HIV Monitor assay, which is based on a reverse-transcription polymerase chain reaction (Roche Molecular Systems, Tokyo, Japan; lower limit of detection, 50 copies/ml). Other laboratory variables were measured using standard methods, as described previously [19].

The electronic medical charts of all subjects were reviewed to determine the presence of comorbidities such as hypertension, diabetes mellitus (DM), and hepatic viral infection. Hypertension was defined as a systolic blood pressure of ≥ 140 mmHg and/or diastolic

blood pressure of ≥ 90 mmHg, or the use of antihypertensive agents at baseline. DM was defined as a diagnosis of DM prior to baseline, or the use of oral antidiabetic agents or insulin at baseline. Hepatitis B virus (HBV) infection was defined as a positive HBV surface antigen test, and hepatitis C virus (HCV) infection was defined as a positive HCV antibody test.

Statistical analysis

All data are expressed as the mean ± standard deviation unless otherwise stated. Common log transformation was applied when data were not normally-distributed. The cumulative incidence of outcomes was estimated using the Kaplan Meier method stratified by the presence and absence of possible risk factors such as elevated serum cystatin C and albuminuria. The log-rank test and the generalized Wilcoxon test were used to analyze differences between curves. Multivariate Cox proportional hazards regression models were used to examine factors associated with the development of cancer and CVD. In addition to cystatin C elevation and albuminuria, the following variables, clinically relevant or previously shown to be risk factors associated with the development of cancer and CVD, were incorporated into the Cox models: age, smoking habit, log CD4 cell counts, eGFR, and CRP level, and coexistence of hypertension, DM, and hepatic viral infection. The hazards ratio (HR) and 95% confidence interval (95% CI) were calculated for each variable. All statistical analyses were conducted using JMP 8.0 (SAS Institute Japan, Tokyo, Japan). Values of $p < 0.05$ were considered statistically significant.

Results

Baseline demographic and clinical characteristics

Table 1 summarizes the baseline demographics and clinical characteristics of the 515 HIV-infected men enrolled in this study. Hypertension and DM were present in 121 subjects (23.5%) and 40 study patients (7.8%), respectively. There were 38 (7.4%) and 24 (4.7%) HBV antigen-positive and HCV antibody-positive patients, respectively. The number of patients in the ACR groups < 10 mg/g, 10 – 19 mg/g, 20 – 29 mg, and ≥ 30 mg/g were 238 (46.2%), 124 (24.1%), 35 (6.8%), and 118 (22.9%), respectively. Elevated serum cystatin C was seen in 42 study participants (8.2%).

Table 1. Baseline demographic and clinical characteristics of HIV-infected men

Patient number	515
Age, years	47.4 ± 11.3
Hypertension (+), no (%)	121 (23.5)
DM (+), no (%)	40 (7.8)
Mean blood pressure, mmHg	95.6 ± 10.0
Smoking (+), no (%)	301 (58.4)
HBV (+), no (%)	38 (7.4)
HCV (+), no (%)	24 (4.7)
Current TDF use, no (%)	279 (54.2)
CD4 cell count, cells/μl	440 ± 207
C-reactive protein, mg/dl	0.39 ± 1.09
Serum creatinine, mg/dl	0.83 ± 0.29
eGFR, ml/min/1.73 m^2	84.7 ± 19.1
ACR, mg/g	128 ± 706
ACR ≥ 30 mg/g, no (%)	118 (22.9)
ACR 20 – 29 mg/g, no (%)	35 (6.8)
ACR 10 – 19 mg/g, no (%)	124 (24.1)
ACR < 10 mg/g, no (%)	238 (46.2)
Serum cystatin C, mg/l	0.80 ± 0.26
Serum cystatin C ≥1.0 mg/l	42 (8.2)

Data are expressed as mean ± standard deviation. Abbreviations: DM, diabetes mellitus; HBV, hepatitis B virus; HCV, hepatitis C; TDF, tenofovir disoproxil fumarate; eGFR, estimated glomerular filtration rate; ACR, urinary albumin-to-creatinine ratio.

Incidence of adverse outcomes during the 3-year follow-up period

All participants completed the 3-year follow-up study. Cancers and CVD developed in 13 (2.5%) and 14 (2.7%), respectively, and 4 died of cancers and 1 of CVD. There were 4 colon cancers, 2 lung, 1 pancreatic, 1 bile duct, 1 oropharyngeal, 1 cancer of the buccal mucosa, 1 testicular, 1 tongue, 1 hepatocellular, and 1 Hodgkin's lymphoma. One patient developed two types of cancers. Among participants with CVD, acute myocardial infarction, cerebral infarction, and heart failure were observed in 7, 4, and 2 subjects, respectively. One subject had both cancer and events associated with CVD.

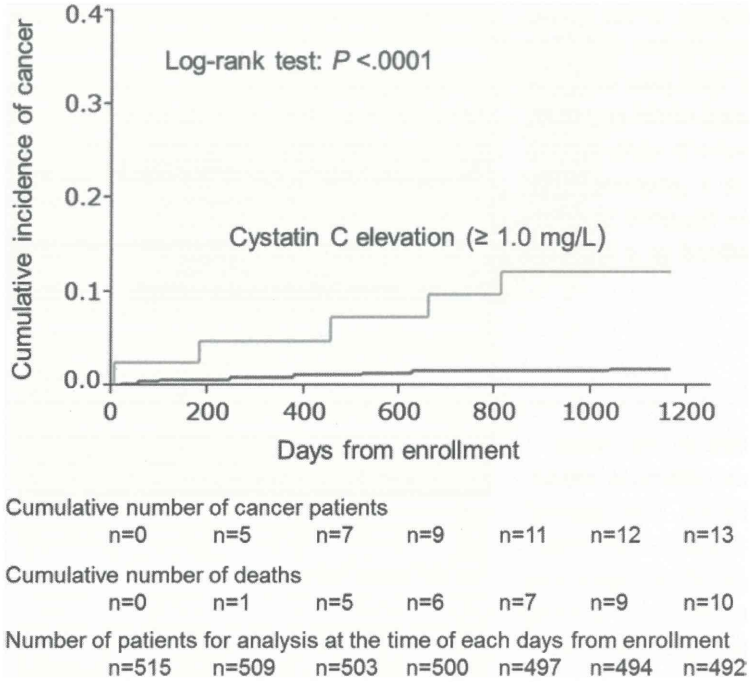

Figure 1. Kaplan-Meier estimate of cumulative incidence of cancer according to presence of cystatin C elevation (≥ 1.0 mg/l) at baseline. The difference between the two groups was statistically significant (log-rank test, p <.0001). Cystatin C elevation was defined as ≥ 1.0 mg/l.

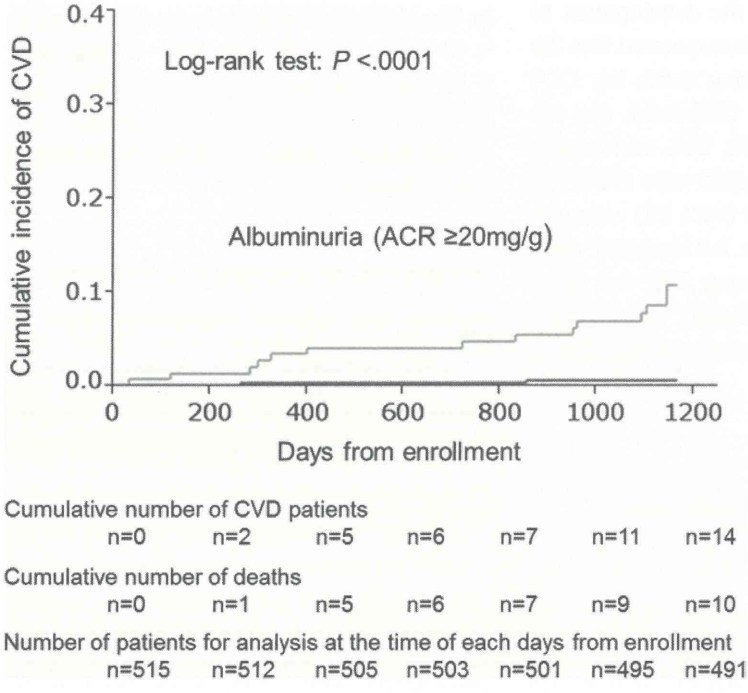

Figure 2. Kaplan-Meier estimate of cumulative incidence of cardiovascular disease (CVD) according to presence of albuminuria (ACR ≥ 20 mg/g) at baseline. The difference between the two groups was statistically significant (log-rank test, p <.0001).

Cumulative incidence of the adverse outcomes

Figures 1 and 2 show Kaplan-Meier curves of the cumulative proportion of patients who developed adverse outcomes, according to the presence or absence of cystatin C elevation (serum cystatin C level ≥ 1.0 mg/l) and albuminuria greater than the high-normal range (ACR ≥ 20 mg/g). The incidence of cancer was significantly increased in patients with cystatin C elevations but not in patients with albuminuria, and the incidence of CVD events was significantly increased in patients with albuminuria but not in patients with cystatin C elevations. The estimates for CVD were also significantly stratified by albuminuria greater than the middle-normal range (ACR ≥ 10 mg/g) and greater than the abnormal range (ACR ≥ 30 mg/g). However, the statistical significance for differences between the 2 Kaplan-Meier curves according to the generalized Wilcoxon test were lower for those cut-off values, compared with the ACR cut-off of ≥ 20 mg/g as follows: χ^2 = 14.0, p = 0.0002 for ACR ≥ 30 mg/g and χ^2 = 6.06, p = 0.0138 for ACR ≥ 10 mg/g versus χ^2 = 24.8, p < 0.0001 for ACR ≥ 20 mg/g.

Association of cystatin C elevation and albuminuria with cancer and CVD

Table 2 lists the variables, including cystatin C elevation and albuminuria and their association with the occurrence of cancer and CVD-associated events. Cystatin C elevation was significantly associated with cancer incidence (HR, 6.09; 95% CI (1.30 – 24.6), p = 0.0234), and albuminuria greater than the high-normal range (ACR ≥ 20 mg/g) with CVD incidence (HR, 8.97; 95% CI (2.19 – 60.8), p = 0.0014). Age was significantly associated with both cancer incidence (HR, 1.11; 95% CI (1.04 – 1.19), p = 0.0003) and CVD incidence (HR, 1.06; 95% CI (1.00 – 1.13), p = 0.0453). The presence of DM at baseline was significantly associated with CVD incidence (HR, 3.78; 95% CI (1.18 – 11.5), p = 0.0257); however, neither albuminuria greater than the abnormal range (ACR ≥ 30 mg/g) or greater than the middle-normal range (ACR ≥ 10 mg/g) was significantly as-

Table 2. Association of cystatin C elevation, albuminuria, and other variables with adverse outcomes in HIV-infected men

	Cancer		CVD	
Variables	HR (95% CI)	p value	HR (95% CI)	p-value
Cystatin C ≥ 1.0 mg/l	6.09 (1.30 – 24.6)	0.0234*	1.17 (0.17 – 6.18)	0.8653
ACR ≥ 20 mg/g	0.58 (0.15 – 1.96)	0.3866	8.97 (2.20 – 60.8)	0.0014*
Age, years	1.11 (1.04 – 1.19)	0.0003*	1.06 (1.00 – 1.13)	0.0453*
Hypertension (+)	0.48 (0.09 – 1.84)	0.3021	0.70 (0.20 – 2.21)	0.5572
DM (+)	0.83 (0.04 – 5.12)	0.8634	3.78 (1.18 – 11.5)	0.0257*
HBV infection (+)	0.05 (0.02 – 4.51)	0.3102	0.013 (0.00 – 4.21)	0.3167
HCV infection (+)	1.64 (0.20 – 8.58)	0.6050	0.02 (0.01 – 1.61)	0.1124
Smoking (+)	0.76 (0.23 – 2.65)	0.6491	1.05 (0.36 – 3.46)	0.9356
Log CD4 cell count, cells/μl	0.46 (0.04 – 6.66)	0.5528	1.56 (0.08 – 42.4)	0.7769
Serum CRP level, mg/dl	1.16 (0.96 – 1.04)	0.5530	0.94 (0.50 – 1.39)	0.7999
eGFR, ml/min/1.73 m^2	1.00 (0.97 – 1.04)	0.8133	1.00 (0.97 – 1.03)	0.8877

CVD = cardiovascular disease, HR = hazard ratio, CI = confidence interval, ACR = urinary albumin-to-creatinine ratio, DM = diabetes mellitus, HBV = hepatitis B virus, HCV = hepatitis C virus, CRP = C-reactive protein, eGFR = estimated glomerular filtration rate.
Asterisk (*) indicates that the variable is significantly associated with the incidence of the outcome. Multivariate Cox proportional hazards regression models were used to examine associations of cystatin C elevation and albuminuria with each outcome. The following covariates were incorporated into the multivariate models in addition to cystatin C elevation and albuminuria: age, smoking habit, log CD4 cell counts, eGFR, CRP, and coexistence of hypertension, DM, and hepatic viral infection.

sociated with CVD incidence, although they were significant in the Kaplan-Meier analysis. The relationships between cystatin C and CVD and between albuminuria and cancer were not significant (data not shown).

Discussion

The highlights of this study include (1) the findings that cystatin C elevation and albuminuria in contemporary HIV-infected men well controlled on HAART were prognostically significant, and (2) the results for albuminuria suggest the need for a reappraisal of the cut-off value used to determine abnormal albuminuria in HIV-infected individuals.

Our study may be the first to suggest that cystatin C elevation is a possible risk for the development of cancers in HIV-infected patients. Elevated serum cystatin C has been considered a predictor of poor prognosis in the general population [12, 13, 20], and Choi et al. [16] have shown that cystatin C is an important risk for mortality among HIV-infected subjects as well. However, those studies focused on the close association between cystatin C and either CVD or CVD-related mortality, but did not evaluate the association between cystatin C and cancer incidence. CKD is a proinflammatory state, and thus may be associated with cancer risk [21]. Cystatin C elevation may reflect the wide spectrum of abnormalities, including the predisposition to inflammation that accompanies renal dysfunction [22]. In addition, some investigators have shown that cystatin C mRNA expression in malignant tumors was increased compared to normal tissues, and that increased extracellular levels of cystatin C correlated significantly with poor outcomes in cancer patients [23, 24]. Our study was performed in a relatively homogeneous population of HIV-infected men with undetectable viral load, because serum cystatin C levels are affected by the replication of HIV [25, 26]. Moreover, the association between elevated cystatin C and cancer incidence was significant by multivariable analysis after an adjustment for serum CRP level, a standard index of systemic inflammation. Taking into consideration all of these findings, cystatin C levels may be of significant value for providing new prognostic information on the development of cancers in HIV-infected individuals.

Periodic monitoring of albuminuria may be important for identifying HIV patients at risk for developing CVD. For this purpose, a

stringent cut-off value of ACR (20 mg/g) that dichotomizes albuminuria between normal and abnormal ranges should be used in an HIV-infected population instead of the current cut-off value. The conventional cut-off is commonly set to be an ACR of 30 mg/g, and clinically significant albuminuria, that is, "microalbuminuria", is usually defined as an ACR of ≥ 30 mg/g for the general population. This cut-off has been employed in numerous studies of HIV-infected individuals as well. For instance, Szczech et al. [27] showed that the prevalence of microalbuminuria in HIV-infected participants is higher than the prevalence in participants without HIV infection, independent of ethnic background. Wyatt et al. [28] showed that microalbuminuria is significantly associated with all-cause mortality among HIV-infected women not receiving antiretroviral therapy. Moreover, Choi et al. [16, 17] demonstrated that the presence of microalbuminuria, even in subjects with an eGFR ≥ 60 ml/min/1.73m^2, was strongly associated with CVD and all-cause mortality in HIV-infected individuals. On the other hand, any degree of albuminuria may have a significant risk for CVD events. Post-hoc analyses of randomized trials in high-risk individuals as well as community-based cohort studies have indicated that increases in albuminuria within the 'normal' range carry increased risks of CVD morbidity and mortality [29, 30, 31, 32]. Ando et al. [33] showed that urinary albumin excretion within the middle-to-high-normal range is an independent risk for near-term development of overt kidney disease in a prospective study of HIV-infected participants. Moreover, our current study also showed that albuminuria, defined as an ACR ≥ 20 mg/g, most significantly stratified HIV patients at risk of CVD, and that it was the only significant cut-off value associated with CVD incidence in the multivariable models adjusted for known major risks of CVD. All these studies on albuminuria probably underscore the clinical importance of monitoring low-grade albuminuria in HIV-infected individuals.

There are several limitations to this study. First, this analysis included only HIV-infected men on HAART protocols whose infections were well controlled, and the results may not be generalizable to women and HAART-untreated men. Second, there are numerous confounding variables that may affect cystatin C and albuminuria, especially DM, hypertension, kidney disease, and use of TDF. It was therefore difficult to account for all of these variables using multivariable analysis. Third, our study had limited power to statistically analyze the relationship between each risk factor and each of the outcomes, i.e., site-specific cancer and CVD, because of the small number of each outcome. Further follow-up studies are warranted concerning this limitation. Last, association between cystatin C and CVD was not obtained, which we think was due to the relatively small sample size of the cohort and short follow-up period.

In conclusion, our study shows that cystatin C elevation and relatively low-grade albuminuria are risks for the occurrence of cancer and CVD, respectively, in well-controlled HIV-infected men on HAART. Monitoring the risk factors may enable us to intervene earlier during the care of HIV-infected patients for the prevention of life-threatening events.

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Research Article Open Access

HIV-Infected Men with an Elevated Level of Serum Cystatin C have a High Likelihood of Developing Cancers

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Abstract

Background: HIV-infected individuals are at high risk for morbidity and mortality regardless of good infection control with highly active antiretroviral therapy (HAART). The residual inflammation after apparent good infection control with HAART may be responsible for an increased risk of mortality including cancers. Serum cystatin C is not only a sensitive marker for renal dysfunction but also a potential marker for inflammation, which may suggest that this marker is something more than a measure of renal function.

Materials and methods: A total of 520 HIV-infected men under good infection control with HAART were enrolled in a 3-year prospective cohort study. The incidence of cancers was investigated with special reference to serum cystatin C level. Cumulative incidence of cancers over time was analyzed by Kaplan-Meier methods. A Cox proportional hazards model was used to calculate the hazard ratio (HR) of developing cancers, adjusted for age, smoking habit, CD4 cell count, serum albumin, estimated glomerular filtration rate below 60 mL/min/1.73m^2, C-reactive protein, and presence of comorbidities including diabetes mellitus, hypertension, and hepatic viral infection.

Results: During the follow-up, cancers developed in 14 (2.7%) subjects. Death occurred in 4 from cancers. The Kaplan-Meier estimate for cancer incidence significantly increased in patients with serum cystatin C elevation (≥ 1.0 mg/L). The HR (95% confidence interval) of cancer incidence was 3.56 (1.08-11.2) for elevation of serum cystatin C, although other markers of inflammation were not significant.

Conclusion: The examination of serum cystatin C may enable earlier recognition of cancers among HIV-infected individuals.

Keywords: Chronic kidney disease; Highly active antiretroviral therapy; Inflammation; Mortality

Introduction

The introduction of highly active antiretroviral therapy (HAART) has markedly reduced AIDS-related death and opportunistic infectious diseases, which had been the main causes of mortality among human immunodeficiency virus (HIV)-infected subjects in the pre-HAART era [1-3]. However, the causes of mortality have shifted to non-AIDS diseases in the contemporary HAART era, most of which are commonly associated with aging [4]. The major causes of death include non-AIDS-defining cancer and cardiovascular disease (CVD), and these deaths are reported to occur at an earlier age in the HIV-infected population than in the general population [5-7]. Previous studies suggest that HIV-infected subjects are at greater risk for morbidity and mortality than HIV-uninfected subjects, partly because HIV-infected subjects often have high prevalence of chronic comorbidities including diabetes mellitus (DM), hypertension, kidney disease, and liver disease [8,9].

The residual immune-insufficiency and inflammation after apparent good infection control with HAART may also be responsible for an increased risk for such age-associated diseases in treated HIV patients, as shown by the prognostic value of the CD4 cell count and other inflammatory markers [10]. Inflammatory markers are elevated in HIV-infected individuals and remain unaltered after HIV-RNA levels are suppressed by HAART [11], which in part may reflect the existence of persistent immune activation [12]. A large body of evidence supports the notion that inflammation plays a role in poor prognosis including cancers in the general population [13-15]. Thus, evaluation of some biomarkers of inflammation may be important for early identification of those at increased risk for critical illness in well-controlled HIV-infected populations.

In general, serum cystatin C level is a well-known biomarker for early kidney disease; however, it may be a clinical marker for the existence of systemic inflammation as well. In fact, serum cystatin C levels increased rapidly after HAART interruption and were correlated with several inflammatory markers [16]. Serum cystatin C provides prognostic information beyond its role as an index of kidney function and may be a better overall measure of the spectrum of pathophysiologic abnormalities [17-19].

Here, we conducted a 3-year prospective cohort study among men under good infection control with HAART to test the impact of elevated serum cystatin C on the development of cancers.

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Materials and Methods

Study design

This study was a prospective cohort study with the aim of ascertaining the relationships between baseline clinical characteristics, laboratory data, and the new onset of cancer during a follow-up of 3 years. The study was performed in accordance with the Declaration of Helsinki and was approved by the institutional review board (approval certificate no. 681-09-1-13). Informed consent was obtained from all participants.

Study population

A total of 520 ambulatory HIV-infected men (mean age: 47.6 ± 11.4) were recruited at the time of routine outpatient HIV care at Tokyo Metropolitan Komagome Hospital and underwent baseline examination between February and April, 2008. Subjects were followed for at least six visits per year over a 3-year period. All subjects received HAART and had undetectable HIV-RNA level (<50 copies/mL) at baseline. 'Having cancer at baseline' was included in an exclusion criterion. No other inclusion and exclusion criteria were set for the study. Virological and immunologic control was well maintained in all subjects throughout the study period. There were no dropouts due to missing data during the follow-up period. The mean duration of follow-up was 3.10 (range, 3.04 - 3.20) years. Tenofovir disoproxil fumarate, abacavir, and zidovudine were used in 278 (53.5%), 98 (18.8%), and 96 (18.5%) subjects, respectively. Likewise, atazanavir/ritonavir, lopinavir/ritonavir, fosamprenavir/ritonavir, and efavirenz were used in 189 (36.3%), 65 (12.5%), 8 (1.5%), and 234 (45.0%) subjects, respectively.

Measurements

Non-fasting blood and urine samples were collected for analysis as part of routine clinical visits. Serum cystatin C and serum creatinine (Cr) was measured in all subjects. Serum cystatin C was measured using the latex agglutination-turbidimetric immunoassay (IATRO Cys-C; Mitsubishi Chemical Medicine Corporation, Tokyo, Japan), with a cut-off value of 1.0 mg/L. Accordingly, serum cystatin C elevation was defined as ≥ 1.0 mg/L. Estimated glomerular filtration rate (eGFR) based on serum Cr was calculated using the 3-variable Japanese equation constructed by the Japanese Society of Nephrology: eGFR (mL/min/1.73 m^2) = 194 × Serum Cr$^{-1.094}$ × Age$^{-0.287}$ × 0.739 (if female) [20]. CD4 cell counts in HIV-infected subjects were determined using a specific monoclonal antibody and fluorescence-activated cell-sorter analysis. HIV-RNA level was measured using the Roche Amplicor HIV Monitor assay based on reverse transcription-polymerase chain reaction (Roche Molecular Systems, Tokyo, Japan; lower detection limit, 50 copies/mL). Other laboratory variables were measured using standard methods as described previously [21].

The electronic medical charts of all subjects were reviewed to determine the presence of comorbidities such as hypertension, DM, and hepatic viral infection. Hypertension was defined as systolic blood pressure of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg, or the use of antihypertensive agents at baseline. DM was defined as a diagnosis of DM prior to baseline, or the use of oral antidiabetic agents or insulin at baseline. Hepatitis B virus (HBV) infection was defined as a positive HBV surface antigen test, and hepatitis C virus (HCV) infection was defined as a positive reactive HCV antibody test.

Statistical analysis

All data are expressed as the mean ± standard deviation unless otherwise stated. Comparisons between 2 groups were performed using the Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. Cumulative curves of incident cancers were prepared by the Kaplan-Meier method, stratified by the presence or absence of serum cystatin C elevation (≥ 1.0 mg/L). The log-rank test was used to analyze difference between the curves. A second analysis was conducted using a Cox proportional hazards regression model to examine the hazard ratio (HR) of developing cancers. For the HR, 95% confidence interval (95% CI) was calculated. The following covariates were evaluated by univariate analysis as they were previously shown or clinically relevant to be risk factors associated with the development of cancer: age, current smoking habit, CD4 cell counts, serum albumin, renal dysfunction (eGFR< 60 mL/min/1.73m^2), C-reactive protein (CRP), and coexistence of DM, hypertension, and hepatic viral infection. Subsequently, a multivariate model included covariates which were set at a value of $P \leq 0.10$ on univariate analysis. All statistical analyses were conducted using JMP 8.0 (SAS Institute Japan, Tokyo, Japan). P values < 0.05 were considered statistically significant.

Results

Baseline demographic and clinical characteristics

Table 1 summarizes the comparative data between patients who developed cancers and those who did not. Hypertension and DM were present in 125 subjects (24.0%) and 41 subjects (7.9%), respectively. HBV antigen-positive and HCV antibody-positive patients were 38 (7.3%) and 24 (4.6%), respectively. A decrease in eGFR below 60 mL/min/1.73 m^2 and serum cystatin C elevation (≥ 1.0 mg/L) were present in 48 (9.2%) and 47 (9.0%), respectively. Mean age was significantly higher in patients with these features than in those without. Differences

	Overall (n=520)	Cancer (+) (n=14)	Cancer (-) (n=506)
Age, years	47.6 ± 11.4	60.6 ± 11.1*	47.2 ± 11.2
Hypertension (+), no. (%)	125 (24.0)	4 (28.6)	121 (24.2)
DM (+), no. (%)	41 (7.9)	1 (7.1)	40 (8.0)
Smoking (+), no. (%)	303 (58.7)	9 (64.3)	294 (58.7)
HBV (+), no. (%)	38 (7.3)	0 (0)	38 (7.6)
HCV (+), no. (%)	24 (4.6)	2 (14.3)	22 (4.4)
CD4 cell count, cells/μL	439 ± 208	364 ± 203	441 ± 208
Serum albumin, g/dL	4.44 ± 0.30	4.15 ± 0.38*	4.44 ± 0.30
Total cholesterol, mg/dL	198 ± 43	169 ± 37*	199 ± 43
eGFR, mL/min/1.73 m^2	84.0 ± 20.5	68.9 ± 28.1*	84.4 ± 20.1
eGFR < 60 mL/min/1.73 m^2	48 (9.2)	3 (21.4)	45 (9.0)
Serum cystatin C ≥ 1.0 mg/L	47 (9.0)	6 (42.9)*	41 (8.2)
Mean serum cystatin C, mg/L	0.80 ± 0.26	1.06 ± 0.78*	0.79 ± 0.23
C-reactive protein, mg/dL	0.41 ± 1.21	1.53 ± 3.49*	0.38 ± 1.07

Data are expressed as mean ± standard deviation.

Abbreviations: no: number; DM: diabetes mellitus; HBV: hepatitis B virus; HCV: hepatitis C virus; eGFR: estimated glomerular filtration rate.

*Asterisk indicates significant difference between the groups with and without cancer (P < 0.01).

Table 1: Baseline demographic and clinical characteristics of HIV-infected men.