Skin Autofluorescence Predicts Cardiovascular Mortality in Patients on Chronic Hemodialysis.

皮膚AGEの蓄積は慢性血液透析患者の心血管死を予測する

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Abstract

Background. Tissue accumulation of advanced glycation end products (AGE) is thought to contribute to the progression of cardiovascular disease (CVD). Skin autofluorescence, a non-invasive measure of AGE accumulation using autofluorescence of the skin under ultraviolet light, has been reported to be an independent predictor of mortality associated with CVD in Caucasian patients on chronic hemodialysis. The aim of this study was to assess the predictive value of skin autofluorescence on all-cause and cardiovascular mortality in non-Caucasian (Japanese) patients on chronic hemodialysis.

Methods. Baseline skin autofluorescence was measured with an autofluorescence reader in 128 non-Caucasian (Japanese) patients on chronic hemodialysis. All-cause and cardiovascular mortality was monitored prospectively during a period of 6 years.

Results. During the follow-up period, 42 of the 128 patients died; 19 of those patients died of CVD. Skin autofluorescence did not have a significant effect on all-cause mortality. However, age, carotid artery intima-media thickness (IMT), serum albumin, high-sensitivity C-reactive protein (hsCRP), skin autofluorescence and pre-existing CVD were significantly correlated with cardiovascular mortality. Multivariate Cox regression analysis showed skin autofluorescence [adjusted hazard ratio (HR) 3.97; 95% confidence interval (CI) 1.67-9.43], serum albumin [adjusted HR 0.05; 95% CI

0.01-0.32], and hsCRP [adjusted HR 1.55; 95% CI 1.18-2.05] to be independent predictors of cardiovascular mortality.

Conclusions. The present study suggests that skin autofluorescence is an independent predictor of cardiovascular mortality in non-Caucasian (Japanese) patients on chronic hemodialysis.

【背景・目的】終末糖化産物(advanced glycation end products (AGE))は糖尿病合併症の原因の一つとされ心血管病の発症・進展にも関与しているが、透析患者では糖尿病の有無にかかわらず血清AGE が高値であることが報告されている。ただし血清AGE 濃度が必ずしも組織のAGE蓄積程度を反映していないとも言われている。一方、皮膚に紫外線を照射しAGE の自然蛍光を評価した値(skin autofluorescence (AF))は、皮膚生検による測定結果との相関が確認されており、白色人種では末期腎不全や糖尿病患者において心血管病および総死亡に対する独立した生命予後予測因子の一つとの報告もある。しかしながら、日本人を含む黄色人種を対象とした縦断的検討の報告はない。そこで我々は皮膚反射率の低い黄色人種の日本人でも皮膚AGE蓄積が心血管死および総死亡と関連するかを検討した。

【方法】対象は2006年7月から2007年6月の期間に皮膚に蓄積したAGEを非侵襲的に測定することが可能であるAutofluorescence reader (AGE reader; DiagnOptics, Groningen, The Netherlands)を用いて皮膚AGE蓄積(Skin AF)を測定した当科関連施設の維持透析患者128名(65.1 ±11.6 歳、男性59 名、透析歴中央値4.0 年、糖尿病34.3%、皮膚反射率が10%未満の症例は除外）。心血管病の既往はSkin AF を測定する以前に心筋梗塞、狭心症、脳卒中(脳梗塞、脳出血も含む)、末梢動脈疾患(Fontaine`s Ⅱ～Ⅳ)と診断をされたものとし、心血管死の定義は死因が心筋

梗塞、心不全、脳卒中、突然死とした。主要エンドポイントは心血管死、副次エンドポイントは総死亡と定義した。Kaplan-Meier法、Log-rank検定およびCox比例ハザード法を用いて解析を行った。

【結果】追跡期間(中央値5.8年)で心血管死の19名を含む42名が死亡した。心血管死群は心血管死に至らなかった群との比較において年齢、intima-media thickness (IMT)、高感度C-reactive protein (CRP)、Skin AFが有意に高値で、心血管病の既往が多く、血清アルブミンは有意に低値であった。ROC 曲線により心血管死に対するSkin AFのカットオフ値は2.58と求められ、これを基準に対象を2 群に分けてKaplan-Meier曲線を描くと2.58以上の群は心血管死が有意に多かった（p＝0.02）。Cox比例ハザード法ではSkin AF(hazard ratio (HR) 3.97, 95% confidence interval (CI) 1.67-9.43, p<0.01）、高感度CRP（HR 1.55, 95%CI 1.18-2.05, p<0.01）、血清アルブミン(HR 0.05, 95%CI 0.01-0.32, p<0.01)が心血管死に対する独立した危険因子であった。総死亡に対しても同様の解析を行ったがSkin AFと総死亡には有意な関連は認められなかった。

【結論】皮膚AGE蓄積は血液透析患者の心血管死を予測する。

Introduction

Advanced glycation end products (AGE) are synthesized by non-enzymatic glycation of proteins and oxidative reactions to form stable structures accumulating on long-lived proteins and to promote cellular stress responses by engagement of the receptor for AGE (1). Accelerated formation and tissue accumulation of AGE on proteins, with slow turnover, occur in patients with chronic, age-related diseases such as diabetes, chronic renal failure, and systemic inflammatory diseases (2, 3).

The accumulation of AGE on tissue proteins has been implicated as a contributing factor in the progression of cardiovascular disease (CVD), which is a major cause of morbidity and high mortality rates in hemodialysis patients (4-7). Tissue accumulation of AGE can be evaluated by skin biopsies, but it requires invasive procedures.

Meerwaldt *et al*. described a non-invasive device, the autofluorescence reader, for measuring AGE accumulation in patients based on skin autofluorescence under ultraviolet light. These authors reported that skin autofluorescence was correlated with collagen-linked fluorescence, pentosidine and N-carboxymethyl lysine (CML) accumulation in the skin, with long-term complications in patients with diabetes; and that it was a strong independent predictor of mortality and associated with CVD in Caucasian patients on chronic hemodialysis (5, 8).

Skin autofluorescence has not been sufficiently evaluated in the non-Caucasian population on chronic hemodialysis in spite of the fact that the incidence of patients with end-stage renal disease (ESRD) treated with dialysis continues to increase worldwide, especially in Asian countries. Moreover, the predictive value of skin autofluorescence on mortality or CVD in non-Caucasian populations with ESRD has not been sufficiently evaluated, although several cross-sectional evaluations have reported that skin autofluorescence is correlated with a history of CVD, high-sensitivity C-reactive protein (hsCRP), aortic stiffness and endothelial progenitor cells in Japanese patients on chronic hemodialysis (9-12). Therefore, the aim of this study was to investigate the predictive value of skin autofluorescence on all-cause and cardiovascular mortality in non-Caucasian (Japanese) patients on chronic hemodialysis.

Materials and Methods

Study design and population

This prospective observational cohort study included 128 stable patients receiving maintenance hemodialysis in the dialysis unit of Fujita General Hospital and Hohrai East Clinic (9). Inclusion criteria were age of 20 years or more, and receiving dialysis treatment for at least 3 months. All patients were non-Caucasian (Japanese), and were

receiving proper nutritional guidance (getting the right amount of calories, protein, fluids, sodium, phosphorus, calcium, potassium, vitamins and minerals). All patients were dialyzed three times weekly for 4 hours with single-use biocompatible synthetic high-flux membranes. The dialysis fluid was bicarbonate dialysate, and the endotoxin level in the dialysate was below the detection limit (0.001 EU/l). Patients with acute/chronic inflammatory disease and active malignancy were excluded. In addition, patients with skin reflectance below 10% were excluded because of the reduced ability of the AGE reader to reliably measure skin autofluorescence in dark skin types (13, 14). This study protocol complied with the Declaration of Helsinki and was approved by the ethics committees at Fukushima Medical University (acceptance no. 762). All patients received an explanation of the procedures and possible risks of this study, and gave written informed consent to participate in this study.

Data Collection

Blood samples were collected just before starting hemodialysis. Serum albumin, hemoglobin, creatinine, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured according to the automated standardized laboratory techniques in the clinical laboratory of each

institution.

The plasma levels of pentosidine and hsCRP were measured at the Mitsubishi Chemical Medience Corporation (Tokyo, Japan). The plasma level of pentosidine was measured by high performance liquid chromatography using a fluorescence detector (Hitachi F-1050; Hitachi, Tokyo, Japan). The plasma level of hsCRP was measured by the nephelometry method using the Behring Nephelometer II (BNII) (Dade Behring, Tokyo, Japan).

Carotid artery intima-media thickness (IMT) was measured via B-mode ultrasonographic scanning using a high-resolution (10 MHz) linear probe (SSD-5000; Aloka, Tokyo, Japan). The carotid artery was scanned at the level of the bifurcation of the common carotid artery and was investigated bilaterally. Three measurements of the IMT on both sides were performed at the site of greatest thickness and two other points (1 cm proximal and 1 cm distal to this site) for each patient. We defined the carotid IMT as the average value of six measurements (three from the right side and three from the left side) for each patient in the study. To exclude interobserver variability, all scans were performed by the same operator. The operator was not blind to the participants' information such as age, medical history, and dialysis duration, but was blinded to the blood sample data and skin autofluorescence data.

Definition

The primary endpoint of this survey was defined as cardiovascular death [consisting of myocardial infarction, congestive heart failure, stroke (hemorrhagic and ischemic), and sudden death], and the secondary endpoint was defined as all-cause death. Outcomes were surveyed every 12 months using the hospital medical records.

The presence of CVD was defined if at least one of the following events occurred before the time of skin autofluorescence measurement: angina pectoris, myocardial infarction, coronary artery surgery, percutaneous coronary intervention, stroke verified by computed tomography (CT), magnetic resonance imaging (MRI), and/or symptoms of neurological disorders, and peripheral artery disease. The definition of peripheral artery disease included patients with intermittent claudication (Fontaine stage II), ischemic rest pain (stage III) or ulcer, necrosis or a history of amputation (stage IV).

Diabetes was defined by glucose values ≥200 mg/dL at any time, fasting glucose values ≥126 mg/dL, or the use of insulin or oral hypoglycemic drugs.

At the end of the follow-up period in March 2012, the survival status of all patients was assessed.

Skin autofluorescence

Skin autofluorescence was assessed by the autofluorescence reader (AGE reader; DiagnOptics, Groningen, The Netherlands) as described previously (15). The measure of autofluorescence was defined as the average light intensity of the excitation spectrum between 420 and 600 nm, divided by the average light intensity of the emission spectrum between 300 and 420 nm, multiplied by one hundred and expressed in arbitrary units (AU). Skin autofluorescence was measured at the volar side of the lower arm, approximately 10-15 cm below the elbow fold, with the patient in a seated position. Autofluorescence was calculated offline by automated analysis and was observer-independent. The intra- and inter-day assay precision expressed as coefficients of variation for autofluorescence reader measurements were 2.5% (N = 10) and 4.6% (N = 12), respectively.

Statistical analysis

All data are presented as means (standard deviation, SD) or medians (interquartile range, IQR), as appropriate for parametric or nonparametric values. Differences between the two groups were calculated using Student's *t*-test or the Mann-Whitney U test, and the χ^2 test was used for categorical differences. The Spearman's rank correlation test was used to estimate the relationships between variables. Patient

survival was assessed using the Kaplan-Meier method. Forward stepwise Cox regression analysis was used to estimate the effect and the 95% confidence interval (CI) of each predictor of all-cause and cardiovascular mortality, both in a univariate and in a multivariate model correcting for all other predictors. All data were statistically analyzed using SPSS (version 20.0, SPSS, Inc., Chicago, IL, USA). Associations with $p < 0.05$ were considered statistically significant.

Results

Baseline investigations

Baseline investigations were summarized in our previous report (Table 1) (9). The mean age of the subjects was 65.1 ± 11.6 years, 46% were male, the median duration of dialysis was 4.0 years (IQR, 2.0-7.0 years), and the weekly dialysis time was 11.4 ± 1.5 hours. Of the 128 patients, 44 were diabetic and 84 were non-diabetic. (Of the 84 non-diabetic patients, causes of end-stage renal disease were classified as follows: 54 patients had primary glomerulonephritis, 16 had hypertension, and 14 had other conditions). In 128 hemodialysis patients, the mean titer of skin autofluorescence was 2.35 ± 0.68 AU, and was significantly increased in diabetic patients compared with non-diabetic patients (2.52 ± 0.69 AU versus 2.27 ± 0.67 AU, $p < 0.01$).

Of the 128 patients, 85 were treated with angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II receptor blocker (ARB). Skin autofluorescence tended to be lower in ACEi and ARB users compared with non-users, the differences of which were not significant ($p = 0.07$).

Correlation between skin autofluorescence and other parameters

Skin autofluorescence correlated with age ($r = 0.32$, $p < 0.01$), pre-existing CVD ($r = 0.30$, $p < 0.01$), diabetes ($r = 0.21$, $p = 0.02$), carotid IMT ($r = 0.22$, $p = 0.02$), plasma pentosidine ($r = 0.20$, $p = 0.03$), and hsCRP ($r = 0.20$, $p = 0.03$); however, gender, body mass index, serum albumin, hemoglobin, creatinine, LDL cholesterol, HDL cholesterol, or triglycerides did not correlate with skin autofluorescence. Dialysis duration ($r = 0.17$, $p = 0.06$) showed a trend for a correlation with skin autofluorescence.

Follow-up

During the follow-up period, 42 of the 128 patients died; of these, 19 patients died of CVD. Of the 19 patients, the classification of cardiovascular death was: myocardial infarction (2 cases), congestive heart failure (13 cases), and stroke (4 cases). Two patients received a kidney transplant and 4 patients transferred to a different hospital

such that we could not follow those patients. The classification of non-cardiovascular death was: neoplasm (9 cases), infection (2 cases), subarachnoid hemorrhage (2 cases), cachexia (4 cases), liver failure (1 case), accident (1 case), traumatic brain hemorrhage (1 case), unknown (1 case) and others (2 cases).

Comparison of baseline data between patients who did and did not reach the primary endpoint

Patients were divided into a primary endpoint patient group and a group who did not reach the primary endpoint (Table 2). Patients who reached the primary endpoint were older, had a higher percentage of pre-existing CVD, a lower serum albumin level and a higher skin autofluorescence level than those who did not reach the primary endpoint.

The cut-off value for cardiovascular mortality was determined by receiver operating characteristics (ROC) analysis (Figure 1). If one chose to maximize both sensitivity and specificity, a cut-off value of 2.58 AU was shown to be the best predictor for cardiovascular mortality, with a sensitivity of 0.667, a specificity of 0.673, and area under the curve (AUC) of 0.693 ($p < 0.01$; 95% CI 0.56-0.83).

Kaplan-Meier curve analysis revealed that cardiovascular mortality was markedly increased in patients with skin autofluorescence values above the cut-off level compared

with patients with values below (Figure 2).

However, skin autofluorescence did not have a significant effect on all-cause mortality when we used both the ROC-derived cut-off value (2.11 AU) and the mean of skin autofluorescence in Kaplan-Meier curve analysis (data not shown).

Based on earlier studies on predictors of cardiovascular mortality (5-9), we selected gender, age, dialysis duration, diabetic status, carotid artery IMT, serum albumin, serum pentosidine, hsCRP, skin autofluorescence and pre-existing CVD as independent variables for modeling. Univariate Cox regression analysis showed that age, carotid artery IMT, serum albumin, hsCRP, pre-existing CVD and skin autofluorescence were predictive markers of cardiovascular mortality. In multivariate Cox regression analysis, skin autofluorescence [adjusted HR 3.97; 95% CI 1.67–9.43], serum albumin [adjusted HR 0.05; 95% CI 0.01–0.32] and hsCRP [adjusted HR 1.55; 95% CI 1.18–2.05] showed independent predictive effects on cardiovascular mortality (Table 3).

Discussion

This study showed that skin autofluorescence is a significant and independent predictor of cardiovascular mortality in non-Caucasian patients on chronic hemodialysis. Most studies about skin autofluorescence have been performed in Caucasian

populations, reporting that skin autofluorescence is correlated with CVD, and is an independent predictor of all-cause and cardiovascular mortality in Caucasian patients with ESRD (5). In non-Caucasian patients with ESRD, although skin autofluorescence has been reported to be associated with the presence of CVD, hsCRP, aortic stiffness, and endothelial progenitor cells (9, 10, 12, 16), the relationship between cardiovascular mortality and skin autofluorescence had not been investigated. This study is therefore the first to show the relationship between skin autofluorescence and cardiovascular mortality in non-Caucasian patients on chronic hemodialysis in a prospective observation.

The values of skin autofluorescence in our patients were lower than those reported by Gerrits *et al* (6). The mean age of our patients was similar to Gerrits *et al*, and on the other hand, percentage of patients with diabetes of Gerrits study was lower than our patients. Taken together, our results suggest that the value of non-Caucasian skin autofluorescence is lower compared with Caucasian value. This might be due to high absorption grade of both excitation and emission light with dark skin type. Difference of reference values between races is clinically important, and this problem need more investigation and is still open to discuss.

Several epidemiological studies have shown that an inflammatory biomarker, the

hsCRP level, is markedly elevated in patients on chronic hemodialysis and is a good predictor of CVD (17). It has also been reported that hsCRP is independently correlated with skin autofluorescence (10). The multivariate analysis in the present study suggested that both skin autofluorescence and hsCRP were independently correlated with cardiovascular mortality.

Age, which was one of the major factors to increase skin autofluorescence and was significantly related to cardiovascular mortality in the univariate analysis, could be a critical confounding factor; however, multivariate analysis revealed that age was not a significant contributing factor for all-cause and cardiovascular mortality. It might be due to the small group size or to the more pronounced role of the other conventional and nonconventional cardiovascular risk factors in hemodialysis patients in general. Therefore, sufficiently-sized prospective investigation and better statistical methods are needed to evaluate.

In the present study, carotid artery IMT tended to be higher ($p = 0.06$) in patients who died in the CVD group compared with carotid artery IMT in patients who did not have fatal cardiovascular events. Carotid artery IMT is a surrogate marker of arteriosclerosis, and its usefulness as an independent prognostic factor has already been reported in both Caucasian and non-Caucasian patients on chronic hemodialysis (16, 18). In the present

study, carotid artery IMT had a significant predictive value for cardiovascular mortality in univariate analysis. However, multivariate Cox regression analysis revealed that carotid artery IMT was not independently related to cardiovascular mortality, probably due to differences in the definition of CVD and the method of carotid artery IMT measurement between researchers, and due to the relatively small sample size.

There is accumulating evidence to show that malnutrition is involved in inflammation and atherosclerosis in chronic kidney disease patients (19). Previous studies have shown that nutritional parameters, such as serum albumin level and/or total cholesterol levels, are the factors related to prognosis, and that serum albumin level is an independent predictor for survival in dialysis patients. In the present study, low serum albumin level was independently correlated with both all-cause and cardiovascular mortality.

Plasma pentosidine levels have been reported to correlate with glomerular filtration rate, and are extremely elevated in uremia; however, plasma pentosidine levels do not reflect tissue AGE levels, and do not predict mortality and CVD in patients on dialysis (20, 21). In fact, in the present study, plasma pentosidine levels did not have any significant effect on all-cause and cardiovascular mortality. Our results indicate that skin autofluorescence is a more useful and relevant biomarker than plasma pentosidine levels for the prognosis of patients on dialysis.

It has been reported that accumulation of chemically stable AGE on long-lived proteins may serve as a measure of cumulative glycemic burden. In diabetes patients, increased level of skin autofluorescence has been reported to be associated with the progression of micro- and macrovascular complications, as well as mortality (8). In the present study, skin autofluorescence had significant correlations with diabetes, and significantly increased in diabetic patients compared with non-diabetic patients.

The present study had several limitations. The AGE reader might be affected by skin color and might not be reliable in patients with very dark skin because of the high absorption grade of the excited light (13, 14). Several studies have demonstrated that skin autofluorescence has the potential to be a useful marker in Japanese patients (11, 12, 22), and the manufacturers of AGE readers have advised that they are developing improvements to the software that will allow measurements in darker skin. Other limitations of the present study were the relatively small sample size; a larger number of events might allow a more powerful multivariate Cox regression model.

Recently, some AGE breakers have been reported to inhibit the development of vascular damage in experimental animals (23, 24). Further studies are necessary to investigate whether treatment to reduce the accumulation of AGE will result in improvement in cardiovascular mortality.

CVD, which is the leading cause of mortality in patients with ESRD, influences both the quality of life of affected patients and the burden of healthcare costs. According to the report from the National Kidney Foundation Task Force on Cardiovascular Disease in Chronic Kidney Disease, ESRD patients on renal replacement therapy had at least a 10- to 30-fold higher risk of cardiovascular mortality compared with an age-, gender- and race-matched control population (25). Early recognition of CVD and risk stratification is crucial to improve the mortality rate; however, there are few prognostic evidences of AGE accumulation in non-Caucasian patients on chronic hemodialysis. Therefore, our study provides further support for the concept of chronic inflammation and oxidative stress as important risk markers of mortality in patients on chronic hemodialysis.

The present study suggests that assessment of the findings provided by autofluorescence readers may have valuable potential for detecting high-risk patients and enabling stratification of cardiovascular risk in non-Caucasian patients on chronic hemodialysis.

Disclosure

None.

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Table 1. Baseline characteristics of hemodialysis patients

Variables	
N	128
Gender (male/female)	59 / 69
Age at baseline (years)	65.1 ± 11.6
Body mass index (kg/m^2)	22.1 ± 3.3
Dialysis duration (years)	4.0 (2.0-7.0)
Pre-existing CVD (%)	39 (30.5)
Diabetes (%)	44 (34.3)
Carotid artery IMT (mm)	0.9 ± 0.4
Albumin (g/dL)	3.7 ± 0.4
Hemoglobin (g/dL)	10.1 ± 1.6
Creatinine (mg/dL)	10.4 ± 3.4
LDL-cholesterol (mg/dL)	81.2 ± 26.6
HDL-cholesterol (mg/dL)	45.5 ± 13.6
Triglyceride (mg/dL)	108.2 ± 61.2
Pentosidine (pmol/mL)	1156 ± 512
High-sensitivity C-reactive protein (mg/L)	1.4 ± 1.7
Skin autofluorescence (AU)	2.35 ± 0.68
ACEi or ARB (%)	85 (66.4)

Values are expressed as mean ± standard deviation or median (interquartile range).

CVD, cardiovascular disease; IMT, intima-media thickness; AU, arbitrary units;

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 2. Comparison of characteristics between patients who did and did not reach the primary endpoint

Variables	Endpoint (-)	Endpoint (+)	p value
N	109	19	
Gender (male/female)	48 / 61	11 / 8	0.26
Age at baseline (years)	63.8 ± 11.4	72.4 ± 9.5	<0.01
Dialysis duration (years)	4.0 (2.0-7.0)	4.0 (2.0-9.0)	0.96
Diabetes (%)	34 (31.2)	10 (52.6)	0.07
Carotid artery IMT (mm)	0.9 ± 0.4	1.1 ± 0.5	0.08
Albumin (g/dL)	3.7 ± 0.4	3.5 ± 0.3	<0.01
Pentosidine (pmol/mL)	1133 ± 521	1291 ± 447	0.24
High-sensitivity C-reactive protein (mg/L)	1.2 ± 1.5	2.9 ± 1.9	<0.01
Skin autofluorescence (AU)	2.29 ± 0.67	2.72 ± 0.6	0.01
Pre-existing CVD (%)	26 (23.9)	13 (68.4)	<0.01
ACEi or ARB (%)	74 (67.9)	11 (57.9)	0.40

Values are expressed as mean ± standard deviation or median (interquartile range).

P values were calculated using Student's *t*-test or the Mann-Whitney U test, and the χ^2 test was used for categorical differences.

IMT, intima-media thickness; AU, arbitrary units; CVD, cardiovascular disease;

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 3. Predictors of cardiovascular mortality by univariate and multivariate Cox regression analysis.

Variables	Univariate		Multivariate	
	HR (95% CI)	p value	HR (95% CI)	p value
Gender (i.e.(male))	1.85 (0.47-4.59)	0.19		NS
Age at baseline (years)	1.09 (1.03-1.14)	<0.01		NS
Dialysis duration (years)	1.00 (0.92-1.09)	0.94		NS
Diabetes	1.79 (0.75-4.31)	0.19		NS
Carotid IMT (mm)	3.15 (1.23-8.10)	0.02		NS
Albumin (g/dL)	0.15 (0.06-0.38)	<0.01	0.05 (0.01-0.32)	<0.01
Pentosidine (pmol/mL)	1.00 (1.00-1.00)	0.30		NS
High-sensitivity C-reactive protein (mg/L)	1.61 (1.27-2.03)	<0.01	1.55 (1.18-2.05)	<0.01
Skin autofluorescence (AU)	2.13 (1.11-4.27)	0.02	3.97 (1.67-9.43)	<0.01
Pre-existing CVD	6.86 (2.60-18.10)	<0.01		NS

HR, hazard ratio; CI, confidence interval; NS, not significant; IMT, intima-media thickness; AU, arbitrary units; CVD, cardiovascular disease.

Figure Legends

Figure 1. Cardiovascular mortality: Receiver operating characteristic curves for skin autofluorescence to discriminate cardiovascular mortality.

Figure 2. Cardiovascular mortality: Kaplan-Meier curves of cardiovascular mortality.

Figure 1

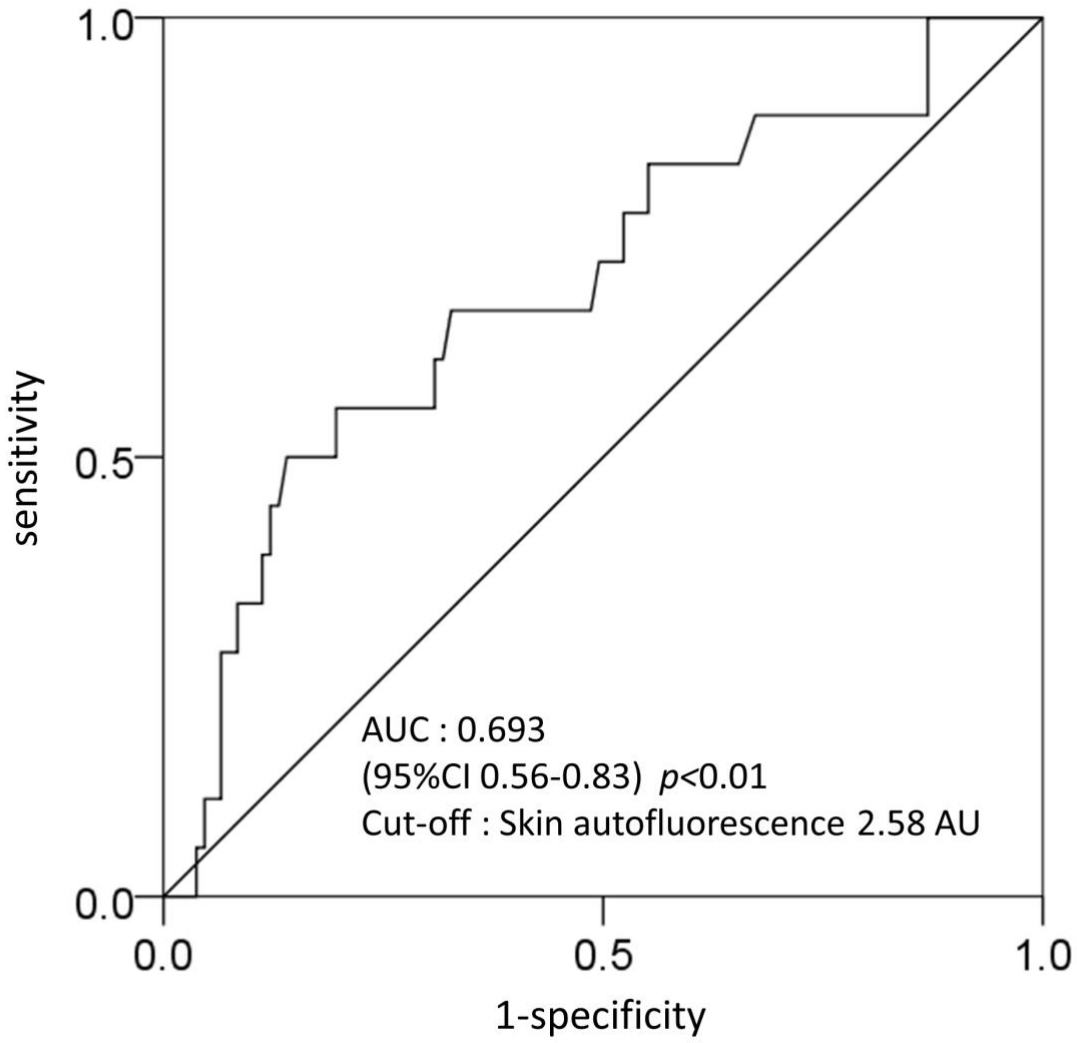
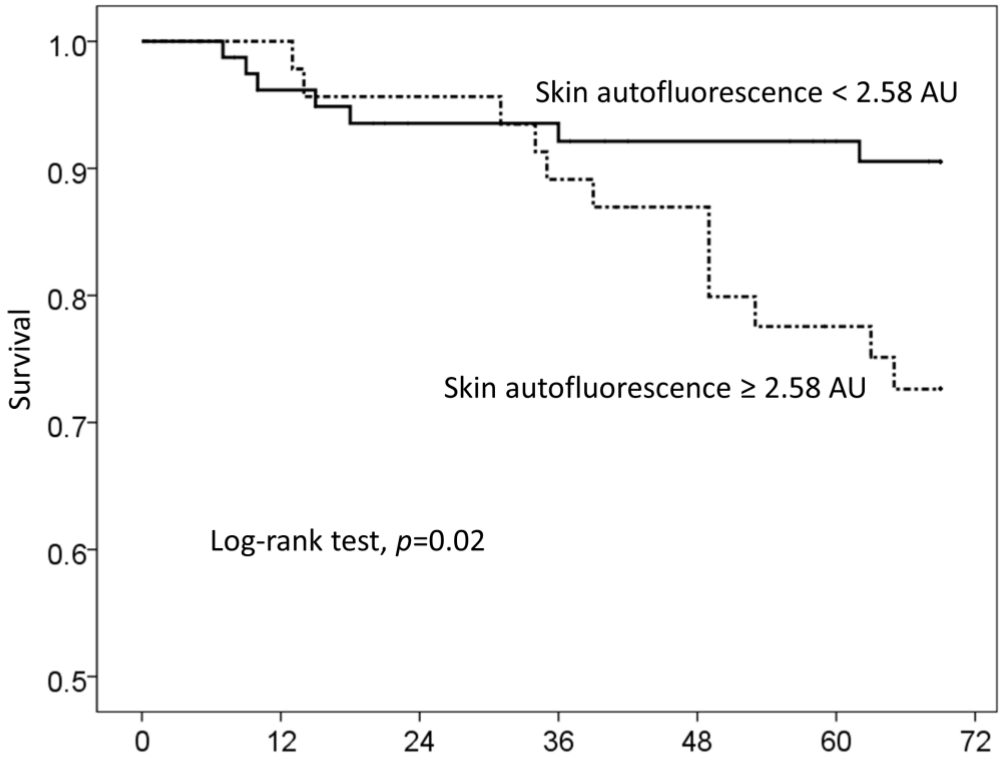

1.0
0.5
0.0
sensitivity
0.0
0.5
1.0
1-specificity
AUC : 0.693
(95%CI 0.56-0.83) p<0.01
Cut-off : Skin autofluorescence 2.58 AU

Figure 2



Number at risk						
<2.58 AU	81	76	73	73	73	73
≥2.58 AU	47	39	35	35	35	35