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Plasma Oxidized Low-Density Lipoprotein as a Prognostic Predictor in Patients With Chronic Congestive Heart Failure

Takashi Tsutsui, MD, Takayoshi Tsutamoto, MD, Atsuyuki Wada, MD, Keiko Maeda, MD, Naoko Mabuchi, MD, Masaru Hayashi, MD, Masato Ohnishi, MD, Masahiko Kinoshita, MD

Otsu, Japan

OBJECTIVES The aim of this study was to evaluate the relationship between plasma oxidized low-density lipoprotein (oxLDL), a marker of oxidative stress, and the prognosis of patients with chronic congestive heart failure (CHF).

BACKGROUND Oxidative stress appears to play a role in the pathophysiology of CHF. We have recently reported the usefulness of plasma oxLDL as a marker of oxidative stress in CHF patients with dilated cardiomyopathy.

METHODS We measured the plasma level of oxLDL by sandwich enzyme-linked immunosorbent assay using a specific monoclonal antibody against oxLDL in 18 age-matched normal subjects and in 84 patients with chronic CHF (New York Heart Association functional class II to IV) and monitored them prospectively for a mean follow-up period of 780 days.

RESULTS Plasma oxLDL level was significantly higher in severe CHF patients than in control subjects and mild CHF patients. A significant negative correlation existed between the plasma level of oxLDL and left ventricular ejection fraction (LVEF) and a significant positive correlation between the plasma level of oxLDL and plasma norepinephrine level. Twenty-six patients had cardiac events; 14 had cardiac death and 12 were hospitalized for heart failure or other cardiovascular events. Among 10 variables including LVEF and neurohumoral factors, only high plasma levels of brain natriuretic peptide and oxLDL were shown to be independent predictors of mortality.

CONCLUSIONS These results indicate that the plasma level of oxLDL is a useful predictor of mortality in patients with CHF, suggesting that oxidative stress plays an important role in the pathophysiology of CHF. (J Am Coll Cardiol 2002;39:957–62) © 2002 by the American College of Cardiology Foundation

Recent studies suggest that free radicals are increased in the failing myocardium and may be important contributors to the deterioration of decompensating myocardium (1–3). A chronic increase in myocardial oxidative stress is capable of causing subcellular abnormalities, and this may lead to cardiomyopathic changes, depressed contractile function and failure (4,5). Thus, oxidative stress may be an important susceptibility factor for congestive heart failure (CHF) (6,7). Several plasma biochemical markers of oxidative stress have been reported to be increased in chronic CHF patients (3,8,9), suggesting that oxidative stress is one of the important mechanisms in CHF progression. Because it is difficult to measure free radicals directly in humans, indirect markers of their activity have been used. Recently, a sensitive and specific method of measuring very low concentrations of oxidized low-density lipoprotein (oxLDL) was established (10,11). We have reported that the plasma level of oxLDL is a useful marker of oxidative stress in the failing heart of CHF patients with dilated cardiomyopathy (DCM) (12), suggesting that left ventricular dysfunction may be partly due to oxidative stress in patients with DCM. However, to our knowledge, there have been no reports about the relationship between plasma markers of oxidative stress and the prognosis of CHF.

Therefore, we evaluated the relationship between the plasma level of oxLDL and the other markers of the severity of CHF, and we assessed whether plasma oxLDL can provide prognostic information independent of clinical and neurohumoral factors previously associated with a poor prognosis in CHF patients.

METHODS

Patients. Eighty-four consecutive patients with mild to severe CHF with DCM or ischemic cardiomyopathy (left ventricular ejection fraction [LVEF] <45%, New York Heart Association [NYHA] functional class II to IV) who visited our hospital between August 1998 and September 1999 were entered into this study. Patients with infection, inflammatory diseases, malignancy, renal failure, congenital malformations of the heart or vessels, angina pectoris or a history of acute myocardial infarction (MI) within the past three months were excluded. The subjects were 63 men and 21 women ranging in age from 17 to 85 years (mean 63.0 ± 1.5 years); 49 patients with ischemic cardiomyopathy had an MI >3 months before the study; 35 had DCM diagnosed as previously reported (12). Fifty patients were classified as NYHA functional class II, 26 patients as class

From the First Department of Internal Medicine, Shiga University of Medical Science, Tsukinowa, Seta, Otsu, Japan. This study was supported by a Japanese Grant-in-Aid for Scientific Research.

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Abbreviations and Acronyms

BNP = brain natriuretic peptide
CHF = congestive heart failure
DCM = dilated cardiomyopathy
HDL = high-density lipoprotein
LVEF = left ventricular ejection fraction
MI = myocardial infarction
NE = norepinephrine
NYHA = New York Heart Association
oxLDL = oxidized low-density lipoprotein
TBARS = thiobarbituric acid-reacting substances

III and 8 patients as class VI. We also selected 18 control subjects (17 to 79 years, mean 58.9 ± 3.1 years) whose hearts were normal on cardiac catheterization and coronary angiography. None of the control subjects had histories of hypertension, diabetes mellitus, hypercholesterolemia or smoking. Informed consent was obtained from all patients before participating in the study, and the protocol was approved by the Human Investigations Committee of our institution.

Study protocol. All patients had rested in bed in the supine position for at least 30 min early in the morning. Blood samples for measuring plasma level of oxLDL, norepinephrine (NE), brain natriuretic peptide (BNP) and serum levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides were collected from the peripheral vein. The LVEF was measured by echocardiography using the biplane disc summation method (Simpson's rule) or left ventriculography in a blinded fashion. Then, the 84 patients were entered and followed prospectively. All surviving patients were monitored prospectively for >589 days, for a mean follow-up period of 780 ± 15.6 days (range 589 to 984 days). The end points, which were judged independently by researchers, were cardiac death (worsening CHF, lethal MI or sudden death) or hospitalization for worsening CHF, MI or fatal arrhythmia.

Measurements of oxLDL, NE and BNP. Blood for measurement of the plasma level of oxLDL was placed into a plain tube, centrifuged at 3,000 rpm for 15 min at 4°C, and the plasma thus obtained was stored at 4°C until assayed. Plasma oxLDL levels were measured using a specific immunometric assay for human oxLDL using a kit (Kyowa Medex, Tokyo, Japan) (10,11), which employed a modification of a method previously reported (10). Briefly, this assay system uses two antibodies against human oxLDL, one recognizing a monoclonal antibody against oxidized phosphatidylcholine (10) and the other a polyclonal antibody against human apolipoprotein B, respectively, and measures oxLDL by sandwiching it between the two antibodies (11,12). Plasma concentration of BNP was measured with a specific immunoradiometric assay using a commercial kit (Shionogi, Osaka, Japan) as previously reported (13). Plasma level of NE was measured with high-performance liquid chromatography as previously reported (13).

Statistical analysis. All results are expressed as the mean ± SEM. Univariate analyses were performed using the Student *t* test. Comparisons between multiple groups were determined by one-way analysis of variance with the Scheffé F test. Categorical data were compared against a chi-square distribution. Linear regression analysis was used to determine the relationship between continuous variables. To ascertain whether measurement of plasma level of oxLDL is useful for predicting morbidity and mortality in patients with chronic CHF, 10 variables were entered into a Cox proportional hazard analysis. Kaplan-Meier analysis was performed on the cumulative cardiac event-free rates in patients with chronic CHF stratified into two groups based on the median plasma oxLDL level. A value of $p < 0.05$ was considered significant.

RESULTS

Clinical characteristics. There was no difference in age, gender or plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol or triglycerides among control subjects, mild CHF patients (NYHA functional class II) and severe CHF patients (NYHA functional class III or IV) (Table 1). The mean LVEF was significantly lower in patients with severe CHF than in patients with mild CHF. There was no difference in etiology and therapy between patients with mild CHF and patients with severe CHF.

Relationship between plasma oxLDL and the severity of CHF. No significant correlation existed between the plasma oxLDL and plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol or triglycerides in control subjects and patients with CHF. The plasma oxLDL level was significantly higher in severe CHF patients than in control subjects and mild CHF patients (Fig. 1). A significant negative correlation was observed between the plasma level of oxLDL and LVEF (Fig. 2A). A significant positive correlation existed between the plasma level of oxLDL and plasma NE level (Fig. 2B).

Characteristics of 84 patients followed with CHF. All of the 84 enrolled patients were successfully followed (Table 2). During the follow-up period, 14 patients died and 12 were hospitalized due to cardiac event. The cause of death was worsening CHF in 12 patients and sudden death in 2 others. The causes of rehospitalization were worsening CHF in 11 patients and fatal arrhythmia in 1 patient. No differences existed between survivors and nonsurvivors by age, gender, etiology, coronary risk factors, LVEF or treatments. Plasma levels of oxLDL, BNP and NE were significantly higher in nonsurvivors than in survivors.

High plasma level of oxLDL as a predictor of morbidity and mortality. By univariate analysis, LVEF, NYHA functional class, plasma levels of BNP, NE and oxLDL were significant predictors of mortality. Stepwise multivariate analysis showed that high plasma levels of both BNP

Table 1. Clinical Characteristics

Characteristics	Control (n = 18)	All Patients (n = 84)	Mild CHF (n = 50)	Severe CHF (n = 34)
Age (yrs)	58.9 ± 3.1	63 ± 1.5	63.8 ± 1.7	61.9 ± 2.8
Gender (M/F)	14/4	63/21	40/10	23/11
HR (beats/min)	67.7 ± 3.5	72.5 ± 1.1	70.2 ± 1.5	76.0 ± 1.7‡
MBP (mm Hg)	80.7 ± 1.3	76.6 ± 0.6†	76.5 ± 0.6‡	76.8 ± 1.1‡
LVEF (%)	66.1 ± 1.5	30.7 ± 1.0*	34.6 ± 1.1*	24.9 ± 1.4*§
LVEDVI (ml/m^2)	85.9 ± 4.8	147 ± 4.4*	130.0 ± 3.4*	171.2 ± 7.9*§
BNP (pg/ml)	12.3 ± 1.4	334 ± 42**	161.5 ± 21.7‡	586.4 ± 82.2*§
NE (pg/ml)	194.4 ± 12.3	592 ± 67.4†	415.3 ± 37.5	850.7 ± 147.3**¶
oxLDL (U/ml)	9.5 ± 0.8	14.3 ± 1‡	11.3 ± 0.9	18.8 ± 1.7**§
TC (mg/dl)	174 ± 5.8	180 ± 4.1	185 ± 5.3	173 ± 6.3
TG (mg/dl)	93 ± 11.8	117 ± 5.7	125 ± 5.7	104 ± 9.9
HDL-C (mg/dl)	44.1 ± 2.9	39.8 ± 1.4	40.8 ± 2.0	38.2 ± 2.0
LDL-C (mg/dl)	111 ± 5.6	114 ± 3.4	114 ± 4.6	114 ± 5.3
Etiology (ICM/DCM)	—	49/35	30/20	19/15
Risk factors				
Diabetes mellitus	—	15 (18%)	10 (20%)	5 (15%)
Hypertension	—	24 (29%)	16 (32%)	8 (24%)
Hypercholesterolemia	—	22 (26%)	15 (30%)	7 (21%)
Smoking	—	16 (19%)	11 (22%)	5 (15%)
Treatments				
Diuretics	—	58 (69%)	33 (66%)	25 (74%)
Digitalis	—	34 (40%)	21 (42%)	13 (38%)
ACE inhibitors or ARB	—	69 (82%)	41 (82%)	28 (82%)
Beta-blockers	—	31 (37%)	22 (44%)	9 (26%)
Vasodilators	—	34 (41%)	18 (36%)	16 (47%)
Statins	—	18 (21%)	13 (26%)	5 (15%)

*$p < 0.0001$ vs. control. **$p < 0.001$ vs. control. †$p < 0.01$ vs. control. ‡$p < 0.05$ vs. control. §$p < 0.0001$ vs. mild. ¶$p < 0.01$ vs. mild.

ACE = angiotensin-converting enzyme; ARB = angiotensin II type 1 receptor blockers; BNP = brain natriuretic peptide; CHF = congestive heart failure; DCM = dilated cardiomyopathy; HDL-C = high-density lipoprotein cholesterol; HR = heart rate; ICM = ischemic cardiomyopathy; LDL-C = low-density lipoprotein cholesterol; LVEDVI = left ventricular end-diastolic volume index; LVEF = left ventricular ejection fraction; MBP = mean blood pressure; NE = norepinephrine; oxLDL = oxidized low-density lipoprotein; TC = total cholesterol; TG = triglycerides.

and oxLDL were significant independent predictors for mortality (Table 3) and also for morbidity and mortality. In patients with plasma oxLDL <12.2 U/ml, survival rates, as determined by Kaplan-Meier analysis, were significantly 1.9 times higher ($p = 0.004$) and the cardiac event-free curve was significantly 2.5 times higher ($p = 0.0017$) (Fig. 3). There was no significant difference in the area under the receiver operating characteristic curves for plasma BNP level and plasma oxLDL level, confirming the equal prognostic values of plasma BNP level and plasma oxLDL level in predicting mortality and morbidity.

DISCUSSION

As a marker of oxidative stress, plasma level of oxLDL was measured using a specific monoclonal antibody against oxLDL in 84 patients with chronic CHF; patients were monitored prospectively. A high plasma level of oxLDL ($p = 0.0006$) was shown to be an important predictor of mortality independent of clinical and neurohumoral factors previously associated with a poor prognosis in patients with CHF. Therefore, we demonstrated that the plasma level of oxLDL is a useful predictor of mortality in patients with CHF, suggesting that oxidative stress plays an important role in the pathophysiology of CHF.

Plasma level of oxLDL increased in patients with CHF. In this study, plasma oxLDL level increased in the peripheral vein in patients with chronic CHF caused by DCM or ischemic cardiomyopathy. The elevated level of plasma oxLDL in the peripheral vein may reflect the increased oxidation status in the failing myocardium or poorly perfused peripheral muscles in patients with CHF. We recently reported that the plasma level of oxLDL measured in the coronary sinus in DCM patients with CHF was significantly higher than that in the aortic root, and there was no difference in the plasma oxLDL level between in the aortic root and in the femoral vein, suggesting that the source of the increase in plasma oxLDL is of myocardial origin rather than peripheral origin. In contrast, thiobarbituric acid-reacting substances (TBARS) measured in the same patients did not show an increase in plasma level as plasma oxLDL did, suggesting that the plasma oxLDL is a sensitive marker of oxidative stress in the heart of DCM patients, and free radicals such as superoxide produced in the failing myocardium may oxidize LDL cholesterol (12).

The plasma level of oxLDL increased with the severity of

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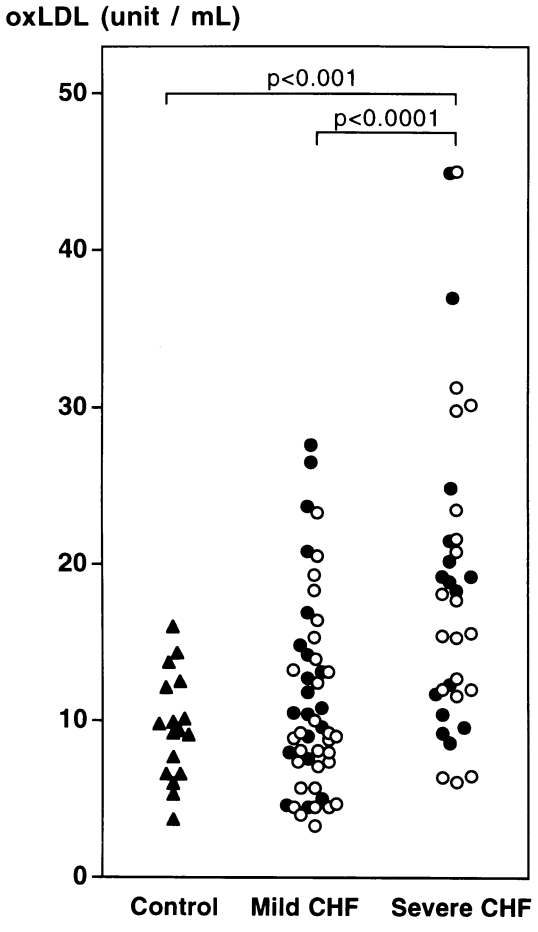

Figure 1. Plasma oxidized low-density lipoprotein (oxLDL) concentration in 18 control subjects, 50 patients with mild congestive heart failure (CHF) (New York Heart Association [NYHA] functional class II) and 34 patients with severe CHF (NYHA functional class III or IV). Mild CHF = NYHA functional class II; severe CHF = NYHA functional class III or IV. **Triangles** = control subjects; **solid circles** = patients with dilated cardiomyopathy; **open circles** = patients with ischemic cardiomyopathy.

CHF in patients with DCM and ischemic cardiomyopathy. In patients with coronary artery disease, the circulating oxLDL may also be derived from coronary atherosclerotic lesions, especially foam-cell-rich unstable plaques in acute coronary syndrome, because it was not increased in patients with stable coronary artery disease (14).

In the present study, plasma oxLDL was measured using the same antibody as the previous study (14), and all 49 patients with ischemic cardiomyopathy had an MI >3 months before the study, and had no clinical signs of ischemia such as chest pain and diagnostic ST-T changes on electrocardiogram by exercise test after MI. Therefore, the increased plasma oxLDL in patients with ischemic cardiomyopathy in the present study was considered mainly due to CHF. Indeed, no ischemic cardiac events were associated with acute coronary syndrome in most patients during the follow-up period.

Plasma level of oxLDL as an independent prognostic predictor in patients with chronic CHF. Increased biochemical markers of oxidative stress measured in peripheral blood and pericardial effusion in patients with CHF have been reported to correlate with functional class (9,15) and inversely with LVEF (3,12). Our findings are consistent with those reports. Hence, it is suggested that oxidative stress is an important factor in the progression of heart failure. However, the relationship between the biochemical markers of oxidative stress and the prognosis of CHF remains unknown. Our study reports for the first time that the plasma level of oxLDL, a clinical marker of oxidative stress, may be a useful prognostic predictor in patients with CHF. In the present study, plasma levels of BNP and NE and low LVEF were prognostic factors in CHF by univariate analysis as previously reported (16,17). However, according to stepwise multivariate analysis, a high plasma level of oxLDL was a significant independent predictor

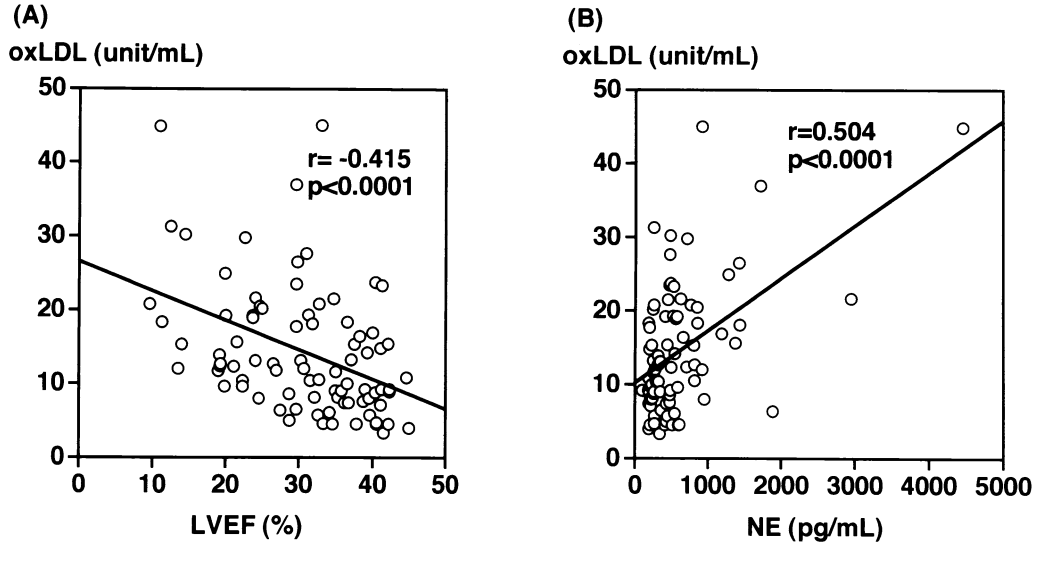

Figure 2. Correlation between the plasma level of oxidized low-density lipoprotein (oxLDL) and left ventricular ejection fraction (LVEF) **(A)**, and the plasma level of norepinephrine (NE) **(B)**.

Table 2. Characteristics of 84 Patients With Chronic Congestive Heart Failure—Survivors and Nonsurvivors With and Without Cardiac Event

Characteristics	Survivors (n = 70)	Nonsurvivors (n = 14)	p Value	Cardiac Event (−) (n = 58)	Cardiac Event (+) (n = 26)	p Value
Age (yrs)	62.3 ± 1.7	66.9 ± 3.2	NS	63.1 ± 1.9	62.9 ± 2.5	NS
Gender (M/F)	54/16	9/5	NS	44/14	19/7	NS
NYHA functional class	2.36 ± 0.07	3.21 ± 0.19	< 0.0001	2.29 ± 0.07	2.96 ± 0.14	< 0.0001
LVEF (%)	31.5 ± 1.1	26.7 ± 2.3	NS	32.8 ± 1.1	25.9 ± 1.6	0.01
oxLDL (U/ml)	12.6 ± 0.8	23.1 ± 3.0	< 0.0001	11.5 ± 0.8	20.5 ± 2.0	< 0.0001
BNP (pg/ml)	240 ± 33	799 ± 141	< 0.0001	195 ± 33	642 ± 90	< 0.0001
NE (pg/ml)	465 ± 37	1227 ± 317	< 0.0001	446 ± 42	915 ± 183	0.001
Etiology (ICM/DCM)	44/26	5/9	NS	37/21	12/14	NS
Risk factors						
Diabetes mellitus	13 (19%)	2 (14%)	NS	12 (21%)	3 (12%)	NS
Hypertension	19 (27%)	5 (36%)	NS	19 (33%)	5 (15%)	NS
Hypercholesterolemia	17 (24%)	5 (36%)	NS	14 (24%)	8 (31%)	NS
Smoking	13 (19%)	3 (21%)	NS	13 (22%)	3 (12%)	NS
Treatments						
Diuretics	48 (69%)	10 (71%)	NS	36 (62%)	22 (85%)	NS
Digitalis	29 (41%)	5 (36%)	NS	23 (40%)	11 (42%)	NS
ACE inhibitors or ARB	58 (83%)	11 (79%)	NS	48 (83%)	21 (81%)	NS
Beta-blockers	27 (39%)	4 (29%)	NS	19 (33%)	12 (46%)	NS
Vasodilators	25 (36%)	9 (64%)	NS	21 (36%)	13 (50%)	NS
Statins	14 (20%)	4 (29%)	NS	12 (21%)	6 (23%)	NS

ACE = angiotensin-converting enzyme; ARB = angiotensin II type 1 receptor blockers; BNP = brain natriuretic peptide; DCM = dilated cardiomyopathy; ICM = ischemic cardiomyopathy; LVEF = left ventricular ejection fraction; NE = norepinephrine; NYHA = New York Heart Association; oxLDL = oxidized low-density lipoprotein.

of mortality, suggesting that oxidative stress is an important prognostic factor in patients with CHF.

Recent reports suggest that myocardial injury caused by free radicals lead to depressed contractile function and myocardial remodeling (4,5). Many factors related to the progression of heart failure, such as neurohumoral factors, including catecholamine (18), angiotensin II (19), cytokines (20) and myocardial mechanical stretch (21), which can induce natriuretic peptide production, are closely linked to free radical formation and oxidative stress at the cellular and molecular levels. Angiotensin-converting enzyme inhibitors and beta-blockers, which have been proven to reduce mortality in patients with chronic CHF, were reported to protect the failing heart partly because of the reduction of myocardial oxidative stress.

Study limitations. In the present study, we did not measure other clinical biochemical markers of oxidative stress. Further studies are needed to determine the comparative prognostic value of other markers; however, plasma oxLDL was a sensitive marker compared with TBARS as previously reported (12). Further studies are needed to clarify the role of repetitive measurement of plasma oxLDL before and after treatments, such as carvedilol, angiotensin-converting enzyme inhibitors, and antioxidants in determining the prognosis of CHF patients.

Conclusions. The plasma level of oxLDL, a marker of oxidative stress, increased with the severity of CHF. A significant correlation existed between the plasma level of oxLDL, LVEF and NE. A high plasma oxLDL level is an independent risk factor for mortality and morbidity in

Table 3. Univariate and Multivariate Predictors of Mortality of 84 Patients With Congestive Heart Failure According to Cardiovascular Death

Variables	Univariate Chi-Square	p Value	Multivariate Chi-Square	p Value
Age (yrs)	1.313	0.2518	0.923	0.3366
Gender (male = 1)	0.975	0.3235	0.007	0.9352
Ischemic etiology (yes = 1)	3.535	0.0601	1.895	0.1686
Diabetes mellitus (yes = 1)	0.132	0.7161	0.572	0.4493
Hypercholesterolemia (yes = 1)	0.857	0.3546	1.881	0.1703
LVEF (%)	4.189	0.0407	0.388	0.5336
NYHA functional class	24.219	< 0.0001	1.299	0.2543
BNP (pg/ml)	36.77	< 0.0001	13.65	0.0002
NE (pg/ml)	42.087	< 0.0001	2.527	0.1119
oxLDL (U/ml)	26.966	< 0.0001	11.787	0.0006

BNP = brain natriuretic peptide; LVEF = left ventricular ejection fraction; NE = norepinephrine; NYHA = New York Heart Association; oxLDL = oxidized low-density lipoprotein.

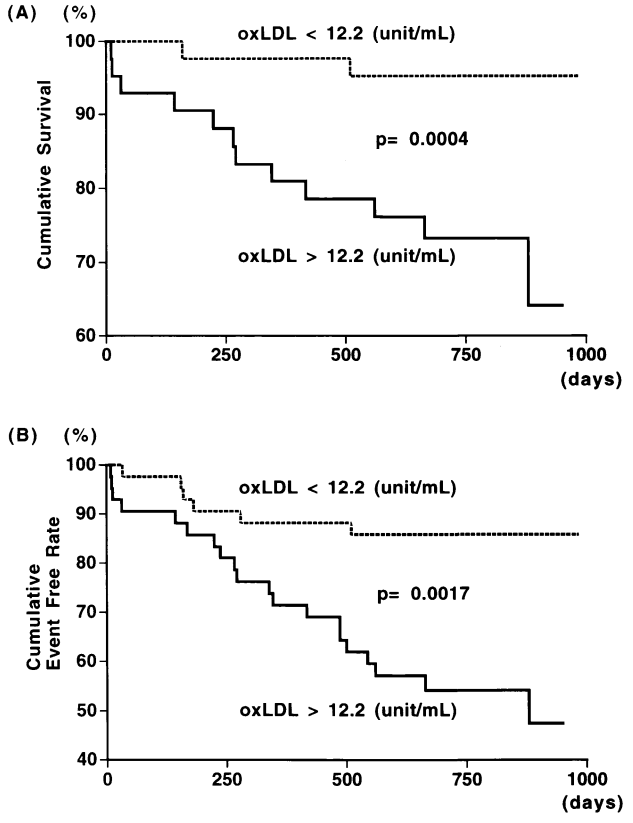

Figure 3. The Kaplan-Meier survival **(A)** and cardiac event-free rate **(B)** plots for 84 patients with congestive heart failure subdivided into two groups according to the median level of oxidized low-density lipoprotein (oxLDL) (12.2 U/ml).

patients with CHF, suggesting that oxidative stress plays an important role in the pathophysiology of CHF.

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Reprint requests and correspondence: Dr. Takayoshi Tsutamoto, First Department of Internal Medicine, Shiga University of Medical Science, Tsukinowa, Seta, Otsu 520-2192, Japan. E-mail: tutamoto@belle.shiga-med.ac.jp.

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