Urinary 8-Hydroxy-2'-Deoxyguanosine as a Novel Biomarker for Predicting Cardiac Events and Evaluating the Effectiveness of Carvedilol Treatment in Patients With Chronic Systolic Heart Failure

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Background: The authors recently reported that urinary 8-hydroxy-2'-deoxyguanosine (U8-OHdG) derived from cardiac tissue reflects clinical status and cardiac dysfunction severity in patients with chronic heart failure (CHF). The aim of the present study was to investigate whether U8-OHdG levels can accurately predict cardiac events in CHF patients and their response to β -blocker treatment.

Methods and Results: Plasma brain natriuretic peptide (BNP) and U8-OHdG levels were measured in 186 consecutive CHF patients before discharge. Patients were then prospectively followed (median follow-up, 649 days) with endpoints of cardiac death or hospitalization due to progressive heart failure. From receiver operating characteristic curve analysis, cut-offs were 12.4 ng/mg creatinine (Cr) for U8-OHdG and 207 pg/ml for BNP. On multivariate Cox analysis, U8-OHdG and BNP were independent predictors of cardiac events. Patients were classified into 4 groups according to U8-OHdG and BNP cut-offs. The hazard ratio for cardiac events in patients with BNP $\geq$ 207 pg/ml and U8-OHdG $\geq$ 12.4 ng/mg Cr was 16.2 compared with approximately 4 for patients with only 1 indicator above its respective cut-off. Furthermore, carvedilol therapy was initiated in 30 CHF patients. In responders ($\geq$ 10% increase in left ventricular ejection fraction [LVEF] or $\geq$ 1 class decrease in New York Heart Association [NYHA] class), U8-OHdG levels decreased significantly along with improved NYHA class, LVEF, and BNP levels after treatment.

Conclusions: U8-OHdG may be a useful biomarker for predicting cardiac events and evaluating β -blocker therapy effectiveness in CHF patients. (*Circ J* 2012; **76**: 117–126)

Key Words: Biomarker; Heart failure; Oxidative stress

Chronic heart failure (CHF) is characterized by hemodynamic abnormalities, neurohormonal activation, relentless progression, and high mortality.^{1,2} CHF progresses through structural remodeling of the heart and likely involves neurohormonal activation.³ Although previous studies have confirmed the prognostic importance of neurohormones such as brain natriuretic peptide (BNP), norepinephrine, interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and angiotensin II (Ang II),^{4–7} BNP has been found to be the most sensitive.³ BNP and N-terminal proBNP are particularly useful for evaluating clinical symptoms and cardiac dysfunction

and predicting prognosis in patients with CHF; this is because they are cardiac specific, because they are secreted from the ventricles according to volume and pressure overload.^{8,9} Thus, they are the most well-established diagnostic and prognostic biomarkers for CHF patients.¹⁰ Recent studies suggest that use of other biomarkers in combination with BNP may exhibit increased effectiveness for evaluating the pathogenesis of heart failure as well as the prognosis and risk stratification of patients with CHF.^{11–13}

Inflammation, activation of the renin-angiotensin-aldosterone system and sympathetic nervous system, and increases in

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Table 1. Baseline Patient Characteristics With and Without Cardiac Events

	Total (n=186)	Event (-) (n=123)	Event (+) (n=63)	P value
Gender (M/F)	114/72	77/46	37/26	0.6356
Age (years)	56.7±14.8	57.1±13.6	55.8±17.0	0.5678
BMI (kg/m ²)	22.5±5.6	22.8±5.5	23.0±5.8	0.1110
NYHA class	2.0±0.8	1.8±0.7	2.5±0.9	<0.0001
U8-OHdG (ng/mg Cr)	13.1±6.0	11.8±5.2	15.7±6.5	<0.0001
U8-isoprostane (ng/mg Cr)	263.4±166.4	235.7±154.3	354.3±184.2	0.8147
Ang II (ng/ml)	25.4±35.3	25.4±39.8	25.5±13.6	0.9941
TNF- α (ng/ml)	2.0±3.2	2.0±3.5	2.0±1.9	0.9793
IL-6 (ng/ml)	4.3±4.5	4.1±4.5	4.6±4.7	0.6446
BNP (pg/ml)	369.7±450.4	268.7±315.9	566.7±590.8	<0.0001
FBS (mg/dl)	99.2±23.7	101.9±24.3	93.4±21.8	0.0707
HbA _{1c} (%)	6.3±1.4	6.4±1.6	6.1±0.9	0.7062
T-cho (mg/dl)	187.4±42.8	190.0±45.1	182.0±37.2	0.3521
Triglycerides (mg/dl)	124.7±67.0	132.5±71.1	101.5±47.4	0.0736
LDL-C (mg/dl)	120.1±40.1	120.9±41.5	116.4±35.3	0.7509
Cr (mg/dl)	1.06±0.52	0.95±0.34	1.28±0.71	0.0067
eGFR (ml/min)	62.7±27.1	66.4±26.2	55.4±27.7	0.0049
Uric acid (mg/dl)	7.2±2.3	6.8±2.0	7.8±2.8	0.0058
TBil (mg/dl)	0.9±0.6	0.9±0.5	0.9±0.6	0.9533
hsCRP (mg/dl)	0.37±0.59	0.32±0.55	0.49±0.66	0.0155
SBP (mmHg)	99.7±17.4	104.0±17.1	90.9±14.5	<0.0001
Heart rate (beats/min)	74.7±13.1	74.9±13.7	74.4±11.8	0.8260
LVDD (mm)	63.3±8.7	62.6±8.7	64.7±8.6	0.1221
LVEDVI (ml/m ²)	128.8±47.3	124.0±45.6	137.1±49.3	0.0793
LVEF (%)	28.5±8.4	29.4±8.2	26.6±8.4	0.0306
Treatment				
β -blocker	83 (155)	80 (98)	90 (57)	0.0651
ACEI/ARB	87 (162)	87 (107)	87 (55)	>0.9999
Loop diuretics	78 (146)	72 (88)	92 (58)	0.0012
Aldosterone antagonist	51 (94)	46 (56)	60 (38)	0.0875
Statin	27 (50)	25 (31)	30 (19)	0.4906

Data given as mean $\pm$ SD or % (n).

BMI, body mass index; NYHA, New York Heart Association; U8-OHdG, urinary 8-OHdG; U8-isoprostane, urinary 8-isoprostane; U8-isoprostane, urinary 8-isoprostane; Ang II, angiotensin-II; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; BNP, B-type natriuretic peptide; FBS, fasting blood sugar; HbA_{1c}, hemoglobin A_{1c}; T-cho, total cholesterol; LDL-C, low-density lipoprotein cholesterol; Cr, serum creatinine; eGFR, estimated glomerular filtration rate; TBil, total bilirubin; hsCRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure; LVDD, left ventricular end-diastolic diameter; LVEDVI, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers.

circulating catecholamine levels and peroxynitrite formed from interactions between superoxide anion and nitric oxide may all increase oxidative stress.¹⁴ Several studies have evaluated the potential utility of plasma myeloperoxidase, plasma oxidized low-density lipoproteins (oxidized LDL), serum/urinary isoprostane, urinary biopyrrins, uric acid, and markers of nitro-redox imbalances such as S-nitrosohemoglobin, nitric oxide, and nitrite for evaluation of CHF severity and prognosis.^{15–23} An oxidized nucleoside of DNA, 8-hydroxy-2'-deoxyguanosine (8-OHdG), is also reported to be a sensitive biomarker for oxidative DNA damage in vivo.^{24–26} It is reported that the production of reactive oxygen species (ROS) results in mitochondrial DNA damage as evaluated with 8-OHdG in mice with failing hearts after myocardial infarction.^{27,28} Furthermore, 8-OHdG can also be detected immunohistochemically in human cardiac tissue in patients with severe dilated cardiomyopathy (DCM).²⁹ Recently, we reported that 8-OHdG is produced in failing cardiac tissue on the basis of sampling

data from the coronary sinus and aortic root.³⁰ In addition, the urinary excretion of 8-OHdG is significantly correlated with clinical status and cardiac dysfunction in patients with CHF.³⁰ Thus, urinary (U) 8-OHdG may be a useful cardiac biomarker for evaluating oxidative stress at the cellular level and predicting the outcome of patients with CHF. In the present study, we investigated whether U8-OHdG is a clinically useful biomarker for predicting the morbidity and mortality of patients with CHF compared with plasma BNP – the current gold standard biomarker for heart failure – and other neurohumoral factors. We also examined whether a combination of plasma BNP and U8-OHdG can reliably be used to stratify the risk of CHF patients for cardiac events.

Methods

Subjects and Study Protocol

One hundred and eighty-six consecutive patients with left ven-

tricular (LV) dysfunction (LV ejection fraction [LVEF] <45%) who had received standard medication, including β -blockers, angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), diuretics, and aldosterone antagonist for >3 months were enrolled in the present study at Yamaguchi University Hospital between June 2005 and June 2010; 135 of these patients were diagnosed with idiopathic DCM; 17 with secondary cardiomyopathy excluding restrictive cardiomyopathy, myocarditis, and cardiac sarcoidosis (ie, hypertensive heart disease, valvular heart disease, or diabetic cardiomyopathy); and 34 with old myocardial infarction (OMI). DCM was diagnosed according to the criteria of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force.³¹ Secondary cardiomyopathy was diagnosed according to clinical history, physical examination, electrocardiogram, echocardiography, and cardiac catheterization including endomyocardial biopsy. OMI was defined as revascularization >3 months previously and no ischemic region in the LV on coronary angiography and/or adenosine-induced myocardial perfusion scintigraphy. All CHF patients were enrolled during steady state within 7 days before discharge. Patients with a smoking habit, acute coronary syndrome, acute myocarditis, severe renal failure, cancer, or inflammatory diseases such as infection or collagen disease were excluded from the study irrespective of CHF severity; this is because oxidative stress has been shown to be increased in these systemic diseases.

Heart failure severity was evaluated on New York Heart Association (NYHA) class, echocardiography, and/or cardiac catheterization; moreover, we collected morning urine samples to measure the concentrations of U8-OHdG and U8-isoprostane as oxidative stress markers and collected blood samples from peripheral veins for blood biochemistry and to evaluate BNP, IL-6, Ang II, TNF- α , and high-sensitivity C-reactive protein (hsCRP) levels. Blood was centrifuged at 3,000 rpm for 15 min at 4°C, and the plasma was frozen in aliquots and stored at -80°C until assaying. Morning urine was also frozen in aliquots and stored at -80°C until assaying. The CHF patients were prospectively followed for a median follow-up period of 649 days, with endpoints of cardiac death or hospitalization due to progressive heart failure.

A total of 186 patients received standard medications, including ACEI, β -blockers, digitalis, and diuretics (Table 1).

Informed consent was obtained from all patients before participation in the study. The protocol was approved by the Human Investigations Committee of Yamaguchi University Hospital and conformed to the Declaration of Helsinki.

Endpoints and Follow-up

No patients were lost to follow-up (median follow-up, 649±505 days) after discharge. Events were centrally adjudicated using medical records, autopsy reports, and death certificates. The first endpoint was defined as cardiac death from worsening heart failure or sudden cardiac death, and the second endpoint was defined as worsening heart failure requiring readmission. Sudden cardiac death was defined as death without definitive premonitory symptoms or signs and was established by the attending physician.

Comparison of U8-OHdG Levels Before and After Carvedilol Treatment

To investigate whether U8-OHdG is useful for evaluating the effectiveness of β -blocker therapy in patients with CHF, 30 consecutive patients who had received standard medication such as diuretics, ACEI/ARB, or aldosterone antagonists, but who had not received β -blocker medications were selected for the present study. A responder was defined as a patient showing clinical improvement of either $\geq 10\%$ in LVEF or ≥ 1 class of NYHA functional classification after treatment with a β -blocker. After treatment with β -blockers (mean follow-up period, 12 months), NYHA class, LVEF on echocardiography, BNP, IL-6, Ang II, TNF- α , hsCRP, and U8-OHdG were re-examined to evaluate their usefulness as oxidative stress biomarkers compared with plasma BNP (the gold standard biomarker of heart failure), and the aforementioned neurohumoral and inflammatory biomarkers.

Measurement of U8-OHdG and Other Neurohumoral and Inflammatory Factors

Serum TNF- α and IL-6 levels were determined using the Quantikine HS (Quantikine HS; R&D Systems, Minneapolis, MN,

Figure 2. Receiver operating characteristic analysis of (A) urinary 8-hydroxy-2'-deoxyguanosine (U8-OHdG) and (B) plasma brain natriuretic peptide (BNP). Kaplan-Meier event-free curves for patients with chronic heart failure divided into 2 groups based on cut-off levels for (C) urinary 8-OHdG and (D) plasma BNP. AUC, area under the curve.

USA) and CLEIA (Fujirebio, Tokyo, Japan) immunoassays.³²

Plasma Ang II levels were measured using a radioimmunoassay with a specific antibody directed against synthetic Ang II as described previously.³³ U8-OHdG levels were measured using a commercially available ELISA kit (Japan Institute for the Control of Aging, Fukuroi, Japan). U8-isoprostane was also determined using an 8-isoprostane EIA kit (Cayman Chemical, Ann Arbor, MI, USA). Raw data were normalized to the urinary level of creatinine (Cr). Plasma BNP was measured using the Shionoria BNP Kit (Shionogi Pharmaceutical, Osaka, Japan). Echocardiographic evaluation was performed by independent echocardiologists. LVEF and cardiac volumes were measured using the Simpson biplane method.

Statistical Analysis

Descriptive statistics for continuous variables are expressed as mean±SD. Student's t-test was used to compare continuous variables between genders. The chi-square test was used to compare prevalence or frequencies. Cut-offs were defined by the point of maximum sensitivity+specificity upon receiver operating characteristic (ROC) analysis. Kaplan-Meier analysis was performed on the cumulative rates of cardiac event-free status in patients with CHF who were divided into 2 groups on the basis of cut-offs for BNP, U8-OHdG, U8-isoprostane, or uric acid. Differences between cardiac event-free curves were analyzed using the log-rank test. To identify independent cardiac event predictors, we initially performed univariate and multivariate analyses with Cox's proportional

Table 2. Risk Factors for Cardiac Events in CHF Patients

	Univariate		Multivariate (model 1)		Multivariate [†] (model 2)	
	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value
Age	0.993 (0.976–1.010)	0.417	0.946 (0.866–1.032)	0.210	0.945 (0.910–0.982)	0.004
Gender	0.828 (0.500–1.372)	0.464	0.148 (0.026–0.855)	0.033		
SBP	0.950 (0.929–0.971)	<0.0001	0.934 (0.886–0.984)	0.011	0.934 (0.902–0.967)	<0.0001
HR	1.000 (0.979–1.021)	1.000	1.033 (0.952–1.119)	0.436		
NYHA						
Grade 1	Reference		Reference			
Grade 2	1.875 (0.845–4.160)	0.122	0.355 (0.041–3.098)	0.349		
Grade 3	4.970 (2.171–11.377)	<0.0001	0.251 (0.013–4.860)	0.360		
Grade 4	13.920 (5.454–35.529)	<0.0001	13.132 (0.069–2,502.927)	0.336		
U8-OHdG	1.071 (1.037–1.106)	<0.0001	1.110 (0.913–1.349)	0.295	1.161 (1.076–1.253)	<0.0001
U8-isoprostane	1.000 (0.998–1.002)	0.947	1.001 (0.996–1.006)	0.704		
BNP	1.001 (1.001–1.002)	<0.0001	1.003 (1.000–1.005)	0.030	1.002 (1.001–1.003)	<0.0001
Ang II	1.001 (0.991–1.011)	0.881	1.006 (0.975–1.038)	0.723		
IL-6	1.042 (0.964–1.126)	0.303	1.247 (0.909–1.711)	0.171		
Uric acid	1.166 (1.057–1.286)	0.002	0.944 (0.670–1.330)	0.741		
CRP	1.354 (0.966–1.898)	0.079	0.957 (0.710–1.289)	0.770		
Sodium	0.824 (0.765–0.888)	<0.0001	1.018 (0.979–1.058)	0.366		
eGFR	0.985 (0.974–0.996)	<0.0001	1.030 (0.996–1.065)	0.082		
LVEDVI	1.004 (0.999–1.009)	0.105	1.104 (0.937–1.302)	0.937		
LVEF	0.968 (0.939–0.998)	0.039	1.030 (0.952–1.302)	0.238		

CHF, chronic heart failure; HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein. Other abbreviations see in Table 1.

[†]Cox proportional model 2 was developed using the stepwise downward method.

hazard model in which we included 16 confounding factors. To refine the models with fewer parameters, we used the step-down method. We adopted Cox's proportional hazard models to estimate hazard ratios (HR) and their 95% confidence intervals for each factor and to adjust for the effects of confounding factors. P-values were 2-sided, and $P<0.05$ was considered significant. Statistical analysis was done using SPSS 16.0J (SPSS Japan, Tokyo, Japan).

Results

Baseline CHF Patient Characteristics

Table 1 summarizes the baseline characteristics of the patients with and without cardiac events. A total of 186 patients with CHF were enrolled in the present study. During the mean follow-up period of 649 days, 63 patients had cardiac events (13 patients died due to worsening heart failure rather than sudden cardiac death, and 50 patients were admitted to hospital for decompensated heart failure; **Figure 1**). There were no differences with respect to gender, age, body mass index, U8-iso-prostane, TNF- α , IL-6, fasting blood sugar, hemoglobin A_{1c}, total cholesterol, triglycerides, LDL cholesterol, total bilirubin, heart rate, LV end-diastolic dimension (LVDD), LV end-diastolic volume index (LVEDVI), or treatment with standard medications other than diuretics between the groups with and without cardiac events. But NYHA class, U8-OHdG, Ang II, plasma BNP, serum Cr, uric acid, and hsCRP were significantly higher and systolic blood pressure (SBP), LVEF, and estimated glomerular filtration rate (eGFR) were significantly lower in the group with cardiac events compared to the group without cardiac events.

Comparison of U8-OHdG and BNP Levels Between Groups

The levels of both U8-OHdG and BNP were significantly higher in patients with cardiac events than in those without (**Table 1**). After dividing the patients with cardiac events into

2 groups (ie, hospitalization and cardiac death), we compared these groups in terms of plasma BNP and U8-OHdG levels to clarify their clinical implications (**Figure 1**). U8-OHdG levels in the cardiac death group were significantly higher than those in the hospitalization group (cardiac death, 19.1 ± 7.4 ng/mg Cr vs. hospitalization, 14.8 ± 6.9 ng/mg Cr, $P=0.0346$), while there was no significant difference in the plasma BNP levels between the 2 groups (cardiac death, 555.0 ± 404.8 pg/ml vs. hospitalization, 569.8 ± 633.7 pg/ml).

Optimal Cut-off Values for Plasma BNP and U8-OHdG

The optimal cut-off values for BNP and U8-OHdG were set at the point of maximum sensitivity plus specificity on each ROC curve. The cut-off level was 12.4 ng/mg Cr for U8-OHdG with a sensitivity of 64.9% and a specificity of 70.4%, and 207 pg/ml for BNP with a sensitivity of 86.0% and a specificity of 59.2% (**Figures 2A,B**).

Univariate and Multivariate Analysis of Cardiac Events

We performed linear univariate and multivariate analyses of cardiac events for 16 variables (age, gender, SBP, heart rate, NYHA, U8-OHdG, U8-isoprostane, BNP, Ang II, IL-6, uric acid, hsCRP, sodium, eGFR, LVEDVI, and LVEF) to identify independent predictors of cardiac events (**Table 2**). Univariate analysis indicated that SBP, NYHA, U8-OHdG, BNP, uric acid, sodium, eGFR, and LVEF were significant predictors. Multivariate analysis identified high levels of SBP ($P<0.0001$), plasma BNP ($P<0.0001$), and U8-OHdG ($P<0.0001$) as significant independent predictors for cardiac events (**Table 2**).

Kaplan-Meier Analysis for Plasma BNP and U8-OHdG

Kaplan-Meier analysis was performed to evaluate the cumulative rates of cardiac event-free status in patients with CHF who were divided into 2 groups on the basis of cut-offs for BNP or U-8-OHdG. It was found that patients with values above the cut-off level had higher cardiac event rates than

Table 3. Clinical Characteristics of the 4 Groups of CHF Patients

	Low U8-OHdG		High U8-OHdG	
	Low BNP	High BNP	Low BNP	High BNP
n	50	57	35	44
Gender (M/F)	28/22	38/19	24/11	24/20
Age (years)	53.7±14.2	56.7±14.5	55.3±15.9	61.2±14.2*
BMI (kg/m ²)	23.9±6.4	22.4±4.7	23.9±7.2	20.0±2.9*†
NYHA class	1.5±0.5	2.0±0.6*	2.0±0.8*	2.7±0.8*†
U8-OHdG (ng/mg Cr)	9.3±2.0	9.1±2.1	17.6±5.0*†	19.2±5.5*†
U8-Isoprostane (ng/mg Cr)	198.2±66.7	228.3±158.9*	307.5±239.8*	480.0±118.6*
Ang II (pg/ml)	21.3±19.3	21.2±29.6	26.4±27.4	41.4±64.1†
TNF- α (pg/ml)	2.6±5.4	1.9±1.9	1.4±0.8	1.8±0.7
IL-6 (pg/ml)	3.4±3.2	5.1±5.6	2.7±1.8	5.8±5.5
BNP (pg/ml)	75.6±54.6	564.6±379.5*	91.7±58.1†	672.3±609.5*
FBS (mg/dl)	103.1±34.9	106.7±42.3	102.3±25.4	96.1±24.7
HbA _{1c} (%)	6.0±0.8	6.9±1.7*	6.3±2.1	5.8±0.7
T-cho (mg/dl)	199.5±42.4	185.3±46.8	172.3±36.8*	185.1±39.0
Triglycerides (mg/dl)	136.7±84.3	109.1±45.3	142.3±73.8	113.8±51.8
Cr (mg/dl)	0.88±0.28	1.05±0.45*	0.95±0.32	1.37±0.77*
eGFR (ml·min ⁻¹ ·1.73 m ⁻²)	70.9±29.1	61.7±24.9	65.8±17.5	52.1±30.8*†
Uric acid (mg/dl)	6.7±1.9	7.4±2.5	6.8±2.3	7.6±2.6
Sodium (mEq/L)	137.9±3.4	137.0±3.7	138.6±2.6	138.4±3.3
T-bil (mg/dl)	0.8±0.4	1.0±0.6	1.0±0.4*	0.9±0.7
hsCRP (mg/dl)	0.16±0.16	0.41±0.55*	0.38±0.80	0.55±0.70*
SBP (mmHg)	107.6±16.2	99.2±19.4*	100.4±11.6*	90.1±15.2*†
Heart rate (beats/min)	70.1±10.6	76.8±13.6*	76.6±14.3*	75.7±13.0*
LVDD (mm)	60.0±7.3	64.8±8.8*	61.4±7.8	64.5±8.3*
LVEF (%)	33.6±7.4	26.7±7.8*	29.8±9.2*	25.4±7.0*
Treatment				
β -blocker	88 (44)	81 (46)	89 (31)	77 (34)
ACEI/ARB	92 (46)	84 (48)	86 (30)	86 (38)
Loop diuretics	74 (37)	79 (45)	74 (26)	86 (38)
Aldosterone antagonist	52 (26)	44 (25)	57 (20)	52 (23)
Statin	26 (13)	21 (12)	37 (13)	27 (12)

Data given as mean ± SD or % (n).

Abbreviations see in Table 1.

*P<0.05 vs. the group with low U8-OHdG (<12.4 ng/mg Cr) and low BNP (<207 pg/ml); †P<0.05 vs. the group with low U8-OHdG (<12.4 ng/mg Cr) and high BNP (≥207 pg/ml).

those below the cut-off (**Figures 2C,D**).

Cardiac Event Prediction Stratified by a Combination of BNP and U8-OHdG

Patients were stratified into 4 groups according to combinations of cut-off levels of BNP and U8-OHdG (**Table 3; Figure 3A**). There were no significant differences in gender among the 4 groups. The HR of patients with high BNP and low U8-OHdG, low BNP and high U8-OHdG, and high BNP and high U8-OHdG were 4.2 (P=0.0101), 4.3 (P=0.0133), and 16.2 (P<0.0001), respectively, compared to those with low BNP and low U8-OHdG (**Figure 3B**).

Effectiveness of Carvedilol in CHF Patients According to U8-OHdG

Table 4 lists the characteristics of the 30 patients with CHF divided into responder and non-responder groups to carvedilol therapy. After carvedilol treatment, heart rate, LVDD, LVEDVI, BNP, and U8-OHdG decreased with an improvement in LVEF or NYHA class in the responder group but not in the non-responder group. In the responders and non-responders, the maximum dosage of carvedilol during the follow-up period

was 11.9±7.1 mg/day and 7.4±6.3 mg/day, respectively. In the non-responders, the dose of carvedilol could not be increased due to adverse effects such as hypotension and worsening cardiac function compared to the responders. In the profiles based on the combined cut-off levels for U8-OHdG and BNP, the statuses of most of the responder patients moved to “low U8-OHdG and low BNP” (**Figure 4A**) after carvedilol treatment, whereas those of most of the non-responder patients did not (**Figure 4B**).

Discussion

The most important finding of the present study is that U8-OHdG is an oxidative stress biomarker that can be used to predict morbidity and mortality; it improves the prediction of cardiac events in combination with plasma BNP in patients with CHF as supported by the following findings. Based on ROC analysis, the cut-off values for U8-OHdG and BNP were determined to be 12.4 ng/mg Cr and 207 pg/ml, respectively. Kaplan-Meier analysis demonstrated that the high U8-OHdG group had a significantly higher incidence of cardiac events than the low U8-OHdG group, as was the case with BNP. Mul-

Table 4. Changes in Clinical Outcomes After Carvedilol Treatment						
	Responder group (n=19)			Non-responder group (n=11)		
	Before	After	P value	Before	After	P value
Age (years)	48±16			55±14		
Gender (M/F)	9/10			6/5		
BMI (kg/m ²)	24.5±7.9			23.1±8.7		
NYHA functional class	2.8±0.4	1.6±0.5	<0.0001	2.5±0.7	3.0±0.9	0.1245
Heart rate (beats/min)	81±17	71±8	0.0325	71±8	74±12	0.6319
SBP	99±22	96±16	0.7638	93.1±11	88±9	0.3293
T-Cho (mg/dl)	182±33	177±30	0.7620	183±31	195±23	0.5697
FBS (mg/dl)	107±57	105±29	0.9449	104±39	114±29	0.6522
LVDD (mm)	66±9	58±7	0.0048	64±7	64±9	0.7951
LVEDVI (ml/m ²)	140±46	105±30	0.0156	143±46	145±59	0.9266
LVEF (%)	23±5	36±13	0.0005	27±10	23±10	0.3847
eGFR (ml·min ⁻¹ ·1.73 m ⁻²)	79±29	69±28	0.3317	60±27	50±28	0.4026
hsCRP (mg/dl)	0.38±0.38	0.14±0.19	0.0734	0.40±0.30	0.51±0.70	0.6859
BNP (pg/ml)	410±295	121±120	0.0003	424±399	571±783	0.5847
U8-OHdG (ng/mg Cr)	15.9±6.9	9.8±3.2	0.0013	13.4±6.3	20.3±4.4	0.0074
Follow-up period (days)		342±340			577±507	
β-blocker dose (mg)		11.9±7.1			7.4±6.3	

Data given as mean ± SD or % (n).
Abbreviations see in Tables 1,2.

Figure 4. Levels of brain natriuretic peptide (BNP) and urinary 8-hydroxy-2'-deoxyguanosine (U8-OHdG) before and after carvedilol treatment in patients with chronic heart failure in the (A) responder and (B) non-responder groups.

tivariate analysis showed that U8-OHdG and BNP were independent predictors of cardiac events. Patients were stratified into 4 groups on the basis of the cut-off levels for both U8-OHdG and BNP (Table 3). Patients exceeding only 1 cut-off level had an HR of 4.2–4.3, while patients exceeding both cut-off levels had an HR of 16.2. Thus, a tandem approach using both BNP and U8-OHdG provides additional risk stratification and a better representation of the status of heart failure by evaluating the aspects of both wall stress and oxidative stress.

U8-OHdG as a Predictor of Cardiac Death in CHF Patients

U8-OHdG levels in the cardiac death group were significantly higher than those in the hospitalization group; meanwhile, there was no difference in plasma BNP levels between the 2 groups (Figure 1). Moreover, in all patients in the cardiac death group, U8-OHdG levels remained high during the fol-

low-up period (data not shown). Thus, a high U8-OHdG level may be a more accurate predictor of cardiac death than BNP level in patients with CHF.

Clinical Implications

There are several advantages of using U8-OHdG as a biomarker for oxidative stress. First, 8-OHdG is partially produced in the failing myocardium;^{27–30} thus, U8-OHdG may closely reflect clinical severity with respect to both symptoms and cardiac dysfunction in CHF.¹⁷ Second, U8-OHdG is predictive of morbidity and mortality in patients with CHF; the HR for cardiac events markedly increased when the cut-off levels for both U8-OHdG and plasma BNP were exceeded.

It is reported that β -blockers and renin-angiotensin-aldosterone blockers both inhibit LV remodeling and improve the prognosis of patients with CHF, partly due to their antioxi-

tive stress effects.^{34–39} Therefore, the present data demonstrate the possibility of treatment guided by both BNP and U8-OHdG as clinical biomarkers (Table 4, Figure 4).

Study Limitations

There were 2 significant limitations of the present study. First, this study was a single-center study; only a small number of subjects were analyzed, and only a small number of cardiac events occurred. A larger study is warranted to confirm the prediction of cardiac events according to U8-OHdG levels in patients with CHF. Second, we observed a consistent change in U8-OHdG levels with respect to the response to carvedilol therapy in 30 CHF patients. We could not clarify, however, whether the antioxidant action was a result of the radical scavenging action of carvedilol or the class effects of β -blockers. Further studies are needed to evaluate the efficacy of other β -blockers such as bisoprolol.

Conclusions

U8-OHdG is a useful oxidative stress biomarker for predicting cardiac events in patients with CHF, and substantially enhances the prediction of cardiac events when used in combination with BNP. Furthermore, U8-OHdG has the potential to be a potent biomarker for evaluating the therapeutic effects of β -blockers.

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Disclosures

No conflict of interest declared.

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