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High Serum Level of Pentosidine, an Advanced Glycation End Product (AGE), is a Risk Factor of Patients with Heart Failure

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ABSTRACT

Background: Pentosidine, one of the advanced glycation end products (AGE), is generated by nonenzymatic glycation and oxidation of proteins. The receptor of AGE (RAGE) is expressed in a variety of tissue, and interaction of AGE with RAGE induces oxidative stress and activation of intracellular signaling causing production of cytokines and mediators of We investigated whether serum pentosidine is a risk factor for heart failure. inflammation. Methods: Serum pentosidine concentration was measured in 141 patients with heart failure and 18 control subjects by a competitive enzyme-linked immunosorbent assay. Patients were prospectively followed during a median follow-up period of 479 days with endpoints of Results: Serum concentration of pentosidine was cardiac death or re-hospitalization. significantly higher in NYHA class III/IV patients than in NYHA class I/II patients (P <0.0001). Serum pentosidine was also higher in patients with cardiac events than in event free patients (P < 0.001). In the univariate Cox proportional hazard analysis, age, NYHA class, pentosidine, creatinine, uric acid, B-type natriuretic peptide, left ventricular end-systolic volume and left ventricular mass were significant risk factors to predict cardiac events. In the multivariate Cox analysis, serum pentosidine concentration was an independent risk factor for cardiac events (hazard ratio 1.88, 95% confidence interval 1.23 - 2.69, P = 0.002).

Patients were divided into 4 groups based on the serum pentosidine levels. The highest 4th quartile of pentosidine was associated with the highest risk of cardiac events (4.52-fold). **Conclusions:** Serum pentosidine concentration is an independent prognostic factor for heart failure, and this new marker may be useful for risk stratification of patients with heart failure.

Key words: pentosidine, advanced glycation end products (AGE), heart failure, prognosis

INTRODUCTION

Heart failure is a growing public health problem, because of the increase of aging population and high prevalence of heart failure in the elderly [1]. A number of studies have demonstrated that inflammation including increased levels of inflammatory cytokines plays a pathological role in heart failure [2]. On the other hand, oxidative stress has been implicated in a wide range of pathological conditions including ischemia/reperfusion injury, degenerative diseases, and aging [3]. Recent experimental and clinical studies have also suggested that oxidative stress increases in heart failure and causes structural and functional disintegrity leading to contractile dysfunction and structural remodeling of the myocardium [4].

Advanced glycation end products (AGE) are generated nonenzymatically by glycation and oxidation. Accumulation of AGE increases with age, in diabetes mellitus [5, 6], and in uremia with or without diabetes [7-9]. Importantly, interaction of AGE with the receptor for AGE (RAGE) causes activation of intracellular signaling, gene expression, production of pro-inflammatory cytokines and free radicals [10]. Although the importance of inflammation and oxidative stress as pathogenesis of heart failure has been widely recognized, clinical significance of the AGE-RAGE system has not been previously examined in heart failure.

Pentosidine, one of well defined AGE, is synthesized through nonenzymatic reactions of pentose, and its formation is closely related to oxidative processes [11]. In the present study, we measured serum pentosidine levels in patients hospitalized for heart failure and examined whether serum pentosidine levels are related to the severity and prognosis in patients with heart failure.

METHODS

Study subjects

We measured serum concentration of pentosidine in 141 patients (88 male and 53 female, mean age 66 ± 13 years) who admitted to the Yamagata University Hospital for diagnosis or treatment of heart failure and 18 age-matched control subjects (8 male and 10 female, mean age 64 ± 13 years). The diagnosis of heart failure was based on a history of dyspnea and symptomatic exercise intolerance with signs of pulmonary congestion or peripheral edema or documentation of left ventricular enlargement or dysfunction by chest X-ray, echocardiography or left ventriculography. The control group was hospitalized in our institution for suspected of coronary artery disease. During hospitalization, the patients were examined by cardiac catheterization, coronary arteriography, echocardiography or blood examination as the need arises. From those examinations, patients with normal coronary arteries and no signs of heart failure were served as control group. We excluded patients with acute coronary syndrome within the 3 months preceding admission, inflammatory diseases and malignant diseases. Written informed consent was obtained from all patients, and Institutional Review Board on human research approved the study protocol.

Baseline clinical characteristics of study subjects are shown in Table 1. The

etiologies of heart failure were identified as ischemic heart failure in 38 patients (27%) and non-ischemic heart failure in the remaining 103 patients (73%). There were 80 patients (57%) with New York Heart Association (NYHA) class I/II and 61 patients (43%) with NYHA class III/IV. Hypertension, diabetes mellitus, hyperlipidemia and current smoking were identified in 83 (59%), 35 (25%), 31 (22%) and 36 (26%) patients, respectively. Hypertension was defined as elevated systolic blood pressure \geq 140 mmHg, diastolic blood pressure \geq 90 mmHg, or when patients had taken antihypertensive drugs. Diabetes mellitus was defined by medical records or by the current use of insulin or oral hypoglycemic medication. Hyperlipidemia was defined by total cholesterol \geq 220 mg/dl, triglyceride \geq 150 mg/dl, high density lipoprotein \leq 40 mg/dl, or current use of antihyperlipidemia drugs. Current smoking was defined by self-report.

Glomerular filtration rate (GFR) was estimated using the Cockcroft-Gault equation [12], which estimates creatinine clearance (ml/min) from serum creatinine and accounts for the effects of age and body weight, as follows: estimated GFR = [(140 - age in years) × (body weight in kilograms)] / (72 × serum creatinine in mg/dl). This value was multiplied by 0.85 in women.

Echocardiography

Echocardiography was performed by an experienced cardiologist within two days after admission. Cardiac function and dimensions were evaluated by two-dimensional echocardiography using a standard technique. Left ventricular end-diastolic diameter (LVEDD), <u>left ventricular end-systole diameter (LVESD)</u>, <u>interventricular septal wall</u> <u>thickness (IVS), left ventricular posterior wall thickness (PW), left ventricular end-diastolic</u> <u>volume (EDV), left ventricular end-systolic volume (ESV), left ventricular mass (LV mass),</u> <u>E/A ratio, deceleration time (DCT)</u> and ejection fraction (EF) were measured.

Measurement of pentosidine

The serum pentosidine concentration in patients with heart failure and control subjects were measured competitive enzyme-linked immunosorbent assay (ELISA, FSK pentosidine ELISA kit, Fushimi Pharmaceutical, Kagawa, Japan). This method involved pretreating sera with a proteolytic enzyme (pronase) and then measuring concentrations of pentosidine in the sample by ELISA [13].

End-points and follow-up

Median follow-up period was 479 days (range 5 to 2553 days). The end points were 1) cardiac death, defined as death from progressive heart failure or sudden cardiac death,

and 2) re-hospitalization due to progressive heart failure [14, 15]. Sudden cardiac death was defined as death without definite premonitory symptoms or signs and was established by the attending physician.

Statistical analysis

All values are expressed as mean \pm SD. A P value less than 0.05 was considered statistically significant. Significance between two groups was determined by unpaired *t* test for continuous variables and chi-square test for discrete variables. The Cox proportional hazard regression model was used to determine which variables were associated with cardiac events. The variables with P values less than 0.05 in the univariate Cox regression analysis were entered into the multivariate Cox regression analysis. Kaplan-Meier survival curve was used to compare cardiac event free rates and analyzed by a log-rank test. All analyses were performed using a Stat View statistical software package (version 5.0, SAS Institute Inc.).

RESULTS

Baseline clinical characteristics of patients with heart failure and control subjects are shown in Table 1. <u>B-type natriuretic peptide (BNP), uric acid, LVEDD, LVESD, IVS, PW,</u> <u>EDV, ESV and LV mass were significantly higher in patients with heart failure than in control subjects.</u> EF was significantly lower in patients with heart failure than in control subjects. <u>As shown in Figure 1, pentosidine concentration was significantly increased in patients with NYHA class III/IV than in control subjects and patients with NYHA class I/II (P<0.05 vs. control and P < 0.001 vs. NYHA I/II).</u>

In heart failure patients, diabetes mellitus was present in 35 patients (25%), and serum pentosidine concentration was not different between diabetic patients and non-diabetic patients (51.2 ± 59.2 vs. 36.4 ± 29.3 ng/ml, P = 0.137). Since it has been reported that serum pentosidine level is increased in renal failure, we examined relationship between serum pentosidine level and renal function. Linear regression analysis demonstrated that serum pentosidine concentration was correlated positively with serum creatinine concentration (R = 0.378, P < 0.0001) and was inversely correlated with estimated GFR (R = -0.334, P < 0.0001).

In patients treated with ACE inhibitors and/or ARB, β -blockers, Ca channel blockers, spironolactone and statins, serum pentosidine levels were not different from patients treated without those drugs. However, in patients treated with diuretics and digoxin, serum pentosidine levels were higher than patients treated without those drugs (diuretics: 58 ± 65 vs. 36 ± 29 ng/ml, P = 0.01; digoxin: 62 ± 66 vs. 42 ± 45 ng/ml, P = 0.04).

During follow-up periods (median 479 days, range 5 to 2533 days), 12 patients died and 20 patients were re-hospitalized due to worsening of heart failure. Table 2 shows comparisons of clinical characteristics between patients with and without cardiac events. Patients with cardiac events (n = 32) were older (P < 0.001) and more frequently had severe NYHA functional class with III/IV (P < 0.0001) than event free patients (n = 109). In patients with cardiac events, levels of serum pentosidine, creatinine, uric acid , BNP, LV mass and E/A were higher than event free patients. Estimate GFR was lower in patients with cardiac events than in event free patients (P < 0.0001). LVEDD, LVESD, IVS, PW, EDV, ESV, DCT and EF were not different between patients with and without cardiac events.

There were 61 patients with EF > 50% out of total 141 patients with heat failure, and these patients were separately analyzed as reported in Table 3. Serum pentosidine level was not significantly different between patients with EF > 50% and patients with EF < 50% (50.8 + 61.7 vs. 47.9 + 47.6 ng/ml, P = 0.765). In 61 patients with EF > 50%, there were 21 patients with NYHA class III/IV, and serum pentosidine level was significantly higher than patients with NYHA class I/II (78.3 + 89.2 vs. 36.3 + 34.0 ng/ml, P = 0.010). Cardiac events occurred in 9 patients among 61 patients with EF > 50%. As shown in Table 3, patients with cardiac events were older than event free patients. Proportion of NYHA class <u>III/IV was higher in patients with cardiac event than event free patients</u>. In patients with EF> 50%, serum pentosidine level was significantly higher in patients with cardiac events than in event free patients (137.7 ± 112.8 vs. 35.7 ± 30.3 ng/ml, P < 0.0001).

To determine risk factors to predict cardiac events, we performed the univariate Cox proportional hazard regression analysis (Table 4). In the univariate analysis, serum pentosidine level was associated with cardiac death and re-hospitalization (per one SD increase, hazard ratio [HR] 1.98, 95% confidence interval [CI] 1.60-2.42, P < 0.0001). Furthermore, age, NYHA functional class, creatinine, uric acid, BNP, estimated GFR, ESV and LV mass were significantly associated with cardiac death and re-hospitalization as shown in Table 4.

Then, those variables with P value of less than 0.05 in the univariate analysis were entered into the multivariate Cox proportional hazard regression analysis. As shown in Table 5, pentosidine and BNP were independent predictors for cardiac events in patients with heart failure (pentosidine: HR 1.60, 95% CI 1.17 – 2.19, P = 0.005 and BNP: HR 2.34, 95% CI 1.00 – 5.36, P = 0.04).

Next, patients were divided into 4 groups based on the pentosidine levels: 1^{st} quartile ($\leq 22.9 \text{ ng/ml}$, n = 35), 2^{nd} quartile (23.0 - 31.9 ng/ml, n = 35), 3^{rd} quartile (32.0 - 46.5 ng/ml, n = 36), and 4^{th} quartile ($46.6 \text{ ng/ml} \leq$, n = 35). In the highest 4^{th} quartile, NYHA functional class was more severe, and levels of creatinine, uric acid and BNP were higher, and estimated GFR was lower than the lower three quartiles (Table 6). As shown in Figure 2, the highest 4^{th} quartile of pentosidine was associated with the highest risk of cardiac events (4.52-fold compared to the lowest 1^{st} quartile). Hazard ratios were 1.00, 1.08 (95% CI 0.290 to 4.025), 1.35 (CI 0.381 to 4.793), and 4.52 (CI 1.521 to 13.439) for quartiles 1 through 4.

The receiver operating characteristic (ROC) curve for pentosidine to predict cardiac events at median follow up period (479 days) is shown in Figure 3. The cut-off value of pentosidine for predicting cardiac events was determined as 41 ng/ml from this ROC curve (sensitivity 62%, specificity 72%). Patients with heart failure were divided into two groups by this cut off value (high pentosidine group, n = 47 and normal pentosidine group, n = 94). In high pentosidine group, there were more patients with NYHA class III/IV than in low pentosidine group (60% vs. 35%, P = 0.006). Cardiac events occurred in 20 patients and 12 patients in high and low pentosidine groups, respectively. As shown in Figure 4, Kaplan-Meier analysis demonstrated that high pentosidine group had a significantly lower cardiac event free rate than low pentosidine group (P = 0.0001 by a log-rank test).

DISCUSSION

In the present study, we showed that serum pentosidine level was higher in patients with severe heart failure with NYHA functional class III/IV than in those with mild heart failure with NYHA class I/II. Pentosidine level was also higher in patients with cardiac events than in those without events. Multivariate Cox proportional hazard analysis demonstrated that pentosidine was the most powerful factor to predict adverse clinical outcomes in patients with heart failure. The highest quartile of pentosidine was associated with the highest risk of cardiac events (4.52-fold compared to the lowest quartile).

Several studies have suggested that pentosidine is accumulated in renal failure [7-9]. In the present study, serum pentosidine concentration was weakly correlated with levels of serum creatinine and estimated GFR (R = 0.378 and R = -0.334, respectively). Previous studies have shown that renal dysfunction, measured by creatinine or estimated GFR, is a strong predictor of mortality in the setting of heart failure [16, 17]. However in the present study, the multivariate Cox hazard analysis showed that creatinine and estimated GFR were not the independent prognostic factor in patients with heart failure.

Although AGEs are generated nonenzymatically by glycation and oxidation, serum pentosidine level was unexpectedly similar between patients with and without diabetes mellitus. Possible reasons for this are 1) most of patients (73%) were non-ischemic heart failure and 2) only 25% of patients had diabetes mellitus in the present study. Furthermore, the presence of diabetes mellitus was not a significant predictor of cardiac events in heart failure by the Cox proportional hazard analysis.

Formation of pentosidine requires oxidation as well as glycation [18, 19]. In the setting of heart failure, excess free-radical generation may arise from many sources. Several clinical studies demonstrated an association between oxidative stress and patients with heart failure [20]. NADPH oxidase activity was increased in the failing myocardium of patients with ischemic cardiomyopathy and dilated cardiomyopathy [21]. Sam F et al. measured directly reactive oxygen species (ROS) in myocardium obtained from patients with dilated cardiomyopathy by electroparamagnetic resonance, and indicated that ROS production was increased in patients with dilated cardiomyopathy [22]. Although AGEs are related with atherosclerosis, the majority of patients in our study have non-ischemic heart disease. Therefore, we could speculate that source of the pentosidine in patients with heart failure might be the heat tissue.

It has been reported that AGE including pentosidine promote oxidative stress and endothelial cell dysfunction [23, 24]. Receptor for AGE (RAGE) is expressed in wide variety of tissues including endothelial cells, smooth muscle cells, cardiac myocytes and macrophages [25, 26]. Expression of RAGE is enhanced in certain cells during diabetes and inflammation. Activation of RAGE by AGEs induces increasing NADPH oxidase activity and production of reactive oxygen species [27]. Interaction of AGE with RAGE causes oxidative stress and activation of nuclear factor (NF)-kB via $p21^{ras}$ and the mitogen activated protein (MAP) kinase signaling pathway [28]. NF-kB modulates gene transcription and generates pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α [29]. Expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) was also enhanced by the AGE-RAGE interaction. These reports support our present findings that patients with high serum levels of pentosidine were associated with high risk for future cardiac events.

In animal experiments, alagebrium that breaks established AGE cross-links between proteins is effective in reducing large artery stiffness, slowing pulse-wave velocity, enhancing cardiac output, and improving left ventricular diastolic distensibility [30-32]. AGE cross-links breaker also improves cardiac function in aging diabetic hearts [33] and in hypertensive hearts [34]. In elderly patients with diastolic heart failure, treatment with alagebrium results in decreases in left ventricular mass and improvements in left ventricular diastolic filling and quality of life [35]. Therefore, these data suggest that the AGE-RAGE may be a new therapeutic target for heart failure.

Conclusions

Serum pentosidine concentration is related to the severity of heart failure and is an independently risk factor to predict adverse clinical outcomes in patients with heart failure. Pentosidine may be a novel marker for risk stratification of patients with heart failure.

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FIGURE LEGENDS

Figure 1. Association between concentration of serum pentosidine and severity of NYHA functional class. * P <0.05 vs. control. #P <0.001 vs. NYHA class I/II.

Figure 2. Association between concentrations of serum pentosidine and cardiac event rates in patients with heart failure. Patients were divided into 4 groups based on the pentosidine levels: 1^{st} quartile ($\leq 22.9 \text{ ng/dl}$, n = 35), 2^{nd} quartile (23.0 - 31.9 ng/dl, n = 35), 3^{rd} quartile (32.0 - 46.5 ng/dl, n = 36), and 4^{th} quartile ($46.6 \text{ ng/dl} \leq$, n = 35). Hazard ratios relative to 1^{st} quartile are shown. * P < 0.01 vs. 1^{st} quartile.

Figure 3. Receiver operating characteristic (ROC) curve analysis to determine optimal sensitivity and specificity. Serum pentosidine level was evaluated for the prediction of cardiac events in patients with heat failure at median follow up periods (479 days).

Figure 4. Kaplan-Meier survival analysis between high and normal pentosidine groups. Patients were divided into two groups based on the cut off value (pentosidine 41 ng/ml) obtained from the ROC curve.

Variables	Control subjects	Heart failure patients P Value		
	(n = 18)	(n = 141)		
Age (y.o.)	64±13	66±13	0.404	
Gender (male/female)	8/10	88/53	0.142	
NYHA class				
NYHA I / II	-	80 (57%)	-	
NYHA III / IV	-	61 (43%)	-	
Etiology				
Ischemic heart failure	-	38 (27%)	-	
Non-ischemic heart failure	-	103 (73%)	-	
Hypertension	1 (6%)	83 (59%)	< 0.0001	
Diabetes mellitus	0 (0%)	35 (25%)	0.017	
Hyperlipidemia	2 (11%)	31 (22%)	0.284	
Current smoking	2 (11%)	36 (26%)	0.177	
Pharmacotherapy				
ACE inhibitors and/or ARBs	1 (6%)	83 (59%)	< 0.0001	
β-blockers	0 (0%)	32 (23%)	0.024	
Ca channel blockers	7 (39%)	38 (27%)	0.290	
Loop diuretics	0 (0%)	75 (53%)	< 0.0001	
Spironolactone	0 (0%)	28 (20%)	0.037	
Digoxin	1 (6%)	41 (29%)	0.033	
Statins	2 (11%)	18 (13%)	0.842	
Laboratory data				
Pentosidine (ng/ml)	33.3±13.2	47.7±52.5	0.250	
Creatinine (mg/dl)	0.70 ± 0.15	$0.96{\pm}0.60$	0.056	
Uric acid (mg/dl)	4.6±1.3	$6.0{\pm}2.2$	0.010	
BNP (pg/ml)	36±37	536±840	0.016	
Estimated GFR (ml/min)	82.5±22.5	69.7±30	0.090	
Echocardiography				
LVEDD (mm)	46±7	53±10	0.004	
EF (%)	67±10	48±20	< 0.001	

Table 1. Clinical characteristics of patients with heart failure and control subjects.

NYHA, New York Heart Association; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; BNP, B-type natriuretic peptide; GFR, glomerular

filtration rate; LVEDD, left ventricular dimension at end-diastole; EF, ejection fraction.

Variables	Cardiac events (-) (n = 109)	Cardiac events (+) (n = 32)	P Value
Age (y.o.)	64±13	73±11	< 0.001
Gender (male/female)	69/40	19/13	0.687
NYHA class			
NYHA I / II	75 (69%)	5 (16%)	
NYHA III / IV	34 (31%)	27 (84%)	< 0.0001
Etiology			
Ischemic heart failure	29 (27%)	9 (28%)	
Non-ischemic heart failure	80 (73%)	23 (72%)	0.865
Hypertension	67 (62%)	16 (50%)	0.246
Diabetes mellitus	29 (27%)	6 (19%)	0.366
Hyperlipidemia	24 (22%)	7 (22%)	0.986
Current smoking	29 (27%)	7 (22%)	0.590
Laboratory data			
Pentosidine (ng/ml)	35.5±25.7	89.1±88.6	< 0.0001
Creatinine (mg/dl)	$0.89{\pm}0.45$	$1.19{\pm}0.80$	0.008
Uric acid (mg/dl)	5.6±1.6	7.2±3.2	< 0.001
BNP (pg/ml)	347±548	1194±1263	< 0.0001
Estimated GFR (ml/min)	75.7±29.5	49.4±25.3	< 0.0001
Echocardiography			
LVEDD (mm)	53±9.6	56±11	0.080
EF (%)	50±20	42±20	0.085

Table 2. Clinical characteristics of patients with cardiac events and events free patients.

Abbreviations as in Table 1.

Variables	chi-square	HR	95% CI of HR	P value
Age (per 1 y.o. increase)	14.1	1.07	1.03 – 1.11	< 0.001
Gender (female vs. male)	0.09	1.11	0.55 - 2.26	0.765
NYHA (class III / IV vs. class I / II)	21.6	9.77	3.73 - 25.6	< 0.0001
Ischemic heart disease	0.001	0.99	0.46 - 2.14	0.971
Hypertension	1.16	0.68	0.34 - 1.37	0.283
Diabetes mellitus	0.39	0.75	0.31 - 1.83	0.533
Hyperlipidemia	0.008	1.04	0.45 - 2.40	0.929
Smoking	0.61	0.72	0.31 - 1.66	0.436
Laboratory data				
Pentosidine (per one SD increase)	38.9	1.98	1.60 - 2.42	< 0.0001
Creatinine (per one SD increase)	20.8	1.89	1.44 - 2.49	< 0.0001
Uric acid (per one SD increase)	19.5	2.35	1.61 - 3.43	< 0.0001
BNP (per one SD increase)	34.6	2.32	2.32 - 2.32	< 0.0001
Estimated GFR (per one SD increase)	21.2	0.34	0.22 - 0.54	< 0.0001
Echocardiography				
LVEDD (per 1 mm increase)	3.68	1.04	1.00 - 1.08	0.055
EF (per 1% increase)	2.71	0.98	0.97 - 1.00	0.100

Table 3. Results of the univariate Cox proportional hazard analysis.

Abbreviations as in Table 1.

HR, hazard ratio; CI, confidence interval

Variables	chi-square	HR	95% CI of HR	P value
Pentosidine (per one SD increase)	8.00	1.60	1.17 – 2.19	0.005
NYHA (class III / IV vs. class I / II)	4.23	3.29	1.06 - 10.3	0.040
Uric acid (per one SD increase)	3.78	1.67	1.00 - 2.79	0.052
Estimated GFR (per one SD increase)	1.26	0.61	0.27 - 1.44	0.262
BNP (per one SD increase)	0.67	1.22	1.00 - 2.31	0.412
Creatinine (per one SD increase)	0.38	0.83	0.45 - 1.51	0.533
Age (per 1 y.o. increase)	0.21	1.16	0.62 - 2.16	0.644

Table 4. Results of the multivariate Cox proportional hazard analysis.

Abbreviations as in Table 1.

HR, hazard ratio; CI, confidence interval

Table 5. Quartiles of serum pentosidine level in patients with heart failure.

	1 st Quartile	2 nd Quartile	3 rd Quartile	4 th Quartile
	(n = 35)	(n = 35)	(n = 36)	(n = 35)
Pentosidine (ng/ml)	<u><</u> 22.9	23.0-31.9	32.0-46.5	<u>></u> 46.6
Cardiac events	4 (11%)	5 (14%)	6 (17%)	17 (49%)§§
Age (y.o.)	65±12	66±15	65±13	70±13
Gender (male/female)	21/14	20/15	26/10	21/14
NYHA class				
NYHA I / II	21 (60%)	24 (69%)	25 (69%)	10 (29%)
NYHA III / IV	14 (40%)	11 (31%)	11 (31%)	25 (71%)§§
Etiology				
Ischemic heart failure	11 (31%)	9 (26%)	10 (28%)	8 (23%)
Non-ischemic heart failure	24 (69%)	26 (74%)	26 (72%)	27 (77%)
Hypertension	19 (54%)	20 (57%)	24 (67%)	20 (57%)
Diabetes mellitus	10 (29%)	6 (17%)	13 (36%)	6 (17%)
Hyperlipidemia	12 (34%)	9 (26%)	5 (14%)	5 (14%)
Current smoking	9 (25%)	12 ((34%)	10 (28%)	5 (14%)
Pharmacotherapy				
ACE inhibitors and/or ARBs	23 (66%)	16 (46%)	25 (72%)	18 (51%)
β-blockers	8 (23%)	9 (26%)	8 (22%)	7 (20%)
Ca channel blockers	11 (31%)	13 (37%)	10 (28%)	4 (11%)
Loop diuretics	18 (51%)	12 (34%)	20 (56%)	25 (71%)§
Spironolactone	8 (23%)	4 (11%)	5 (14%)	11 (31%)
Digoxin	6 (17%)	7 (20%)	13 (36%)	15 (43%)§
Statins	7 (20%)	4 (11%)	3 (8%)	4 (11%)
Laboratory data				
Creatinine (mg/dl)	0.82 ± 0.17	0.80±0.23	0.96 ± 0.64	1.25±0.81*##
Uric acid (mg/dl)	5.41±1.75	5.71±1.74	5.86 ± 1.75	7.01±3.04*
BNP (pg/ml)	431±618	344±495	380±537	1037±1329*##†
Estimated GFR (ml/min)	80.0±29.5	78.7±31.5	70.9 ± 25.9	49.4±26.0**##†
Echocardiography				
LVEDD (mm)	55±7.1	50±11	55±11	54±9.1
EF (%)	48±19	54±21	46±19	45±20

Abbreviations as in Table 1.

* P <0.05 vs. 1st quartile, †P <0.05 vs. 3rd quartile, ** P <0.01 vs. 1st quartile, ## P <0.01 vs. 2nd quartile. § P <0.05, §§ P < 0.01



Figure. 1

*



Figure. 2





Follow up periods (days)

Figure. 4