Serum cortisol as a useful predictor of cardiac events in patients with chronic heart failure - the impact of oxidative stress

Yamaji

Running title: cortisol and oxLDL as a cardiac event predictor

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ABSTRACT

Background. The pathophysiological role of cortisol, which binds to the mineralocorticoid receptor with an affinity equal to that of aldosterone (ALD), may be influenced by oxidative stress in CHF patients. We evaluated cardiac event prediction using cortisol levels in chronic heart failure (CHF), comparison with ALD, adrenocorticotropin hormone (ACTH) and brain natriuretic peptide (BNP), and the impact of oxidative stress.

Methods and Results. We measured the plasma levels of biomarkers such as BNP, ALD, ACTH, serum cortisol and oxidized low density lipoprotein (oxLDL), a biomarker of oxidative stress, in 319 consecutive symptomatic CHF patients and then we prospectively followed these patients for a mean period of 33 months. During the follow-up period, 29 patients had cardiac events (death or hospitalization). Plasma levels of BNP, ALD, ACTH, oxLDL and serum cortisol (16.8±1.8 vs. 12.4±0.3μg/dL, p=0.01) were significantly higher in patients with cardiac events than in those without cardiac events. On stepwise multivariate analyses, high levels of BNP (p=0.0003), renin (p=0.002), cortisol (p=0.02) and oxLDL (p=0.002) were independent predictors of
cardiac events, but ALD and ACTH levels were not. In patients with serum cortisol >12.5μg/dL, the hazard ratio of cardiac events in patients with oxLDL >12unit/mL was 5.0 compared with that in patients with oxLDL <12unit/mL (p=0.004).

Conclusions. These findings indicate that serum cortisol levels were a complementary and incremental cardiac event risk predictor in combination with BNP in CHF patient and that cardiac event prediction based on cortisol levels was influenced by oxidative stress.

Key words: cortisol, aldosterone, BNP, oxidative stress, cardiac events
Suppression of increases in the renin – angiotensin-aldosterone system (RAAS) is a cornerstone in the management of chronic heart failure (CHF) patients and monitoring of RAAS activity might be useful for assessing CHF severity and response to therapy 1-6. Higher plasma aldosterone (ALD) concentrations were associated with increased mortality and rehospitalization rates 4, 5, 7. A previous report suggested that mineralocorticoid receptor (MR) antagonist decreased BNP and improved left ventricular remodeling 8, and in RALES and EPHESUS study, MR antagonist improved survival and lowered hospitalization in patients with severe CHF and post myocardial infarction 9,10.

MR binds mineralocorticoids and glucocorticoids with equal affinity 11,12. Circulating glucocorticoid concentrations are 100- to 1,000- fold higher in plasma compared to those of ALD and thus, in principle would be expected to preferentially occupy the MR. However, the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), which converts cortisol to the non-MR binding metabolite cortisone, allows ALD to selectively bind and activate MR 13-15. MR is expressed in the heart 16 and is up-regulated in CHF 17,18. However, 11βHSD2 levels in the heart are almost negligible.
Therefore, it has been proposed that cardiac MR are occupied by cortisol rather than ALD 18-20.

Yet, cortisol usually acts as an MR antagonist in the kidney and heart 21, 22. If an intracellular redox state changes with tissue damage and generation of reactive oxygen species (ROS) in CHF, cortisol may act as MR agonist like ALD 18-20, 23. However, the usefulness of predicting cardiac events based on cortisol levels, which may also bind and activate the MR, remains unclear. Güder et al. reported that higher serum levels of both cortisol and aldosterone were independent predictors of increased mortality risk in CHF patients 24. However, they did not evaluate BNP, adrenocorticotropic hormone (ACTH) and a biomarker of oxidative stress.

The present study evaluated the cardiac events prediction based on serum cortisol levels in CHF patients, in comparison with that based on ALD and ACTH, which stimulates the secretion of ALD and cortisol, and in combination with BNP, which is a well-established prognostic predictor. Furthermore, we assessed the impact of oxidative stress on the prognostic role of cortisol, which has been reported in relation to cortisol-MR complex activation 18-20, 23.
Methods

Patients

The study population was drawn from 520 consecutive symptomatic CHF patients admitted to our hospital between 2003 and 2008 for management of CHF. Among these patients, the study population consisted of 319 consecutive CHF patients under cardiac catheterization for clinical indication several days after the management of CHF. We prospectively followed these patients for a mean period of 33 months and cardiac events were defined as cardiac death (including sudden death) or hospitalization for CHF. Patients with acute myocardial infarction, congenital heart disease, renal failure (serum creatinine > 2.5 mg/dl), or malignancy were excluded. New York Heart Association (NYHA) functional class was evaluated on the day of cardiac catheterization. Informed consent was obtained from each patient for participation in the study, according to a protocol approved by the institutional Committee on Human Investigation.

Study Protocol

All patients were performed cardiac catheterization. Hemodynamic measurements and blood samples for measuring the plasma levels of neurohumoral factors [i.e., BNP,
norepinephrine (NE), plasma renin concentration (PRC), angiotensin II and ALD, ACTH, oxidized low density lipoprotein (oxLDL), serum levels of cortisol, sodium and creatinine and other laboratory data were collected from a peripheral vein prior to the procedure after at least 20 min of supine bed rest. Blood was centrifuged at 4°C, and then the plasma was frozen in aliquots and stored at –30°C until assay. The plasma levels of BNP, NE, PRC, angiotensin II, and ALD were measured as previously reported. The plasma oxLDL levels were measured by specific immunoradiometric assay using a commercial kit (Kyowa Medex, Tokyo, Japan), as previously reported. The serum cortisol levels and plasma ACTH levels were measured using a commercial radioimmunoassay kit (SRL, Tokyo, Japan). Left ventriculography was performed using contrast medium or radioisotope after hemodynamic measurements and blood sampling.

Oxidative stress was estimated by oxLDL and oxLDL > 12unit/mL was defined as positive as previously reported. This cut-off level was calculated by receiver operating characteristics (ROC) analysis to detect cardiac events. The attending physicians were unaware of the neurohumoral data.

Statistical analysis
All results are expressed as the mean± SEM. Categorical variables were presented by frequency counts and percentages, and intergroup comparisons were analyzed by χ²–test. Univariate analyses were performed using Student’s t-test. Because BNP, NE, PRC, angiotensin II, ALD, ACTH and cortisol levels were not normally distributed, differences between the groups were detected by Mann-Whitney U-test with 2-tailed p-values <0.05. Variables with non-normal distribution were transformed logarithmically before linear regression analysis. Associations between serum cortisol levels and measurement variables were examined through Pearson’s correlation coefficient and linear regression. Multivariate linear regression analyses were performed to examine the independent correlates of log-transformed cortisol levels, including baseline variables that were associated with cortisol levels at p-values < 0.10 on univariate analysis. The β-coefficients were standardized regression coefficients. The prediction of cardiac events was tested by Cox proportional hazards regression analysis with categorized variables dichotomized at the cut-off levels. The cut-off level was defined by the maximal point of (sensitivity + specificity) on ROC analysis. Multivariate Cox proportional hazards analyses were performed as stepwise regressions and the main models were adjusted by
variables that were considered traditional risk factors for mortality in CHF and that were associated with cardiac events at p-values < 0.10 on univariate analysis in the present study. The sensitivity and specificity of the cut-off levels of BNP, NE, PRC, ALD and cortisol for predicting cardiac event risk were determined by ROC analysis.

Kaplan-Meier analysis was performed on the cumulative rates of cardiac event-free status in patients with CHF divided into 2 groups based on cut-off levels of BNP, NE, PRC, ALD and cortisol, and differences between the cardiac event-free curves were analyzed by log-rank test. The cardiac event prediction utility of the combination of cortisol and BNP, oxLDL was analyzed by Cox proportional hazards regression analysis.

A value of p < 0.05 was considered significant.

**Results**

**Clinical characteristics (Table 1)**

Patients were divided into 2 groups: with and without cardiac events. Twenty-nine patients had cardiac events (17 patients died due to worsening heart failure or sudden death and 12 patients were admitted to hospital for decompensated heart failure) during the mean follow-up period of 33 months. Neurohumoral data such as BNP, NE, PRC,
angiotensin II, ALD, ACTH, cortisol and oxLDL levels were significantly higher in patients with cardiac events than in those without cardiac events.

**Univariate and multivariate predictors of cardiac events (Table 2)**

Patients with CHF were divided into 2 groups based on cut-off levels for each variable calculated by ROC analysis to detect cardiac events and cardiac event prediction was evaluated by Cox proportional regression analysis. The cut-off level was determined as 187pg/mL for BNP, giving a sensitivity of 65.5% and specificity of 67.2%, 378pg/mL for NE, giving a sensitivity of 65.5% and specificity of 66.5%, 54pg/mL for PRC, giving a sensitivity of 69% and specificity of 72.8%, 89pg/mL for ALD, giving a sensitivity of 58.6% and specificity of 77.7%, and 12.5µg/dL for cortisol, giving a sensitivity of 82.8% and specificity of 52.1%. Kaplan-Meier analysis was performed on the cumulative rates of cardiac event-free status in CHF patients divided into 2 groups based on cut-off levels of BNP, NE, PRC, ALD and cortisol. Patients with higher plasma BNP, NE, PRC, ALD and cortisol showed a high risk of cardiac events (Figure 1).

On stepwise multivariate analyses, only high levels of plasma BNP (p=0.0003), PRC (p=0.002), serum cortisol (p=0.02) and oxLDL (p=0.002), but plasma ALD and ACTH levels were not, were significant independent predictors of cardiac events (Table
2). Hazard ratios of cardiac events for neurohumoral factors adjusted for age, sex and BMI were shown in Figure 2.

**Cardiac event prediction stratified by the combination of BNP and cortisol**

Patients were stratified into 4 groups based on cut-off levels of the combination of BNP and cortisol (Figure 3). Compared with patients who had low levels of both BNP and cortisol, patients with high levels of both variables had a 15.5-fold higher cardiac event risk (p=0.0003). Furthermore, in patients with plasma BNP > 187 pg/mL the hazard ratio of cardiac events in patients with serum cortisol > 12.5 µg/dL was 3.4 compared with that in patients with serum cortisol < 12.5 µg/dL (p=0.04).

**Influence of oxidative stress**

Patients were further stratified into 4 groups based on cut-off levels of the combination of cortisol and oxLDL (Figure 4). Even though among patients with serum cortisol > 12.5 µg/dL, none of the patients with oxLDL < 12 unit/mL showed significant prediction of cardiac events compared with the patients with serum cortisol < 12.5 µg/dL. However, in patients with serum cortisol > 12.5 µg/dL, the hazard ratio of cardiac events in patients with oxLDL > 12 unit/mL was 5.2 compared with that in patients with oxLDL < 12 unit/mL (p=0.004).
Univariate and multivariate linear regression model of serum log cortisol (Table 3)

On univariate analysis, 11 clinical, neurohumoral and hemodynamic variables were significant predictors of high serum log cortisol. On stepwise multivariate analyses, 3 parameters such as plasma levels of NE, ALD and ACTH were significant independent predictors.

Effect of spironolactone

Spironolactone was more frequently prescribed for patients with cardiac events than for those without cardiac events (Table 1); probably because patients receiving spironolactone had more severe CHF. The mean dose of spironolactone did not significantly differ between patients with and without cardiac events (26.9±2.4 vs. 25.8±1.6mg/day, p=0.76). Plasma ALD levels were significantly higher in patients receiving spironolactone than in those not receiving spironolactone (93.8±6.1 vs. 55.2±2.9pg/mL, p<0.0001) and serum cortisol levels were slightly higher in patients receiving spironolactone than in those not receiving spironolactone (13.4±0.6 vs. 12.4±0.4μg/dL, p=0.18), but the difference was not significant. Further, intake of spironolactone was significantly associated with plasma ALD levels (r= 0.315, p<0.0001) but not with cortisol levels (r= 0.08, p=0.18). Moreover, spironolactone
administration to patients was a significant predictor of cardiac events on univariate analysis (p=0.002), but not on multivariate analysis.

**Discussion**

**MR activation by cortisol**

Cortisol usually acts as an MR antagonist in the kidney and heart \(^{21, 22}\). Funder et al. indicated that if an intracellular redox state changes with tissue damage and generation of ROS, cortisol may act as an MR agonist like ALD \(^{19, 20, 23}\). In CHF patients, intracellular NADP oxidase is activated and generation of ROS is increased in cardiomyocytes \(^{27}\), and then cortisol may act as an MR agonist under those conditions. In the present study, high serum cortisol was an independent predictor of cardiac events. However, there was no significant difference in the prediction of cardiac events between patients with high-cortisol and low-oxidative stress and those in whom both variables were low (p=0.13) and only patients with high levels of both cortisol and oxidative stress showed a significantly higher risk of cardiac events (p=0.004) (Figure 4). These findings suggested that oxidative stress was required in order for cortisol to be cardiac event predictor and might provide one explanation of the phenomenon that oxidative stress activates the cortisol-MR complex as an MR agonist and serum cortisol levels had a
cardiac event prediction value.

Moreover, in the present study, there was a close correlation between serum cortisol levels and plasma ACTH levels (Table 3), which are considered a nonspecific indicator of stress, and these levels were higher in patients with cardiac events than in those without cardiac events. Both cortisol and ACTH levels were significant predictors of cardiac events on univariate analysis (Table 2). However, on multivariate analysis, only serum cortisol levels were an independent predictor of cardiac events, but plasma ACTH levels were not (Figure 2), suggesting that cortisol is not only a nonspecific indicator of stress but may also increase the risk of cardiac events by acting as an MR agonist.

Predictor of cardiac events

In the present study, BNP and PRC were significant independent predictors of cardiac events in CHF patients, consistent with previous reports. Furthermore, the present study indicated that serum cortisol levels were an independent predictor of cardiac events even after adjusting for traditional risk factors, but plasma ALD levels were not (Figure 2), which was not consistent with the findings in a previous study. The prognostic importance of BNP, a biomarker of ventricular wall stress, has been
confirmed by many clinical studies as previously reported. Although CHF patients with higher ALD levels showed a high risk of cardiac events (Figure 1) and ALD levels were positively associated with cardiac event risk with a HR of 4.08 (95% CI, 1.36 to 12.2) for the highest versus lowest quartile of ALD (data not shown) in the present study, the prognostic role of ALD remains controversial because many factors, including therapy for heart failure, influence the plasma levels of that marker. Especially, MR blockers may increase plasma ALD levels and actually plasma ALD levels were significantly higher in patients receiving spironolactone than in those not receiving spironolactone in the present study. Therefore, plasma ALD levels may not be appropriate for assessing RAAS activity in CHF patients receiving standard therapy.

In RALES and EPHESUS, although MR antagonist reduced mortality and the rate of sudden death in patients with severe CHF and post myocardial infarction, ALD levels were within the normal range and salt status was unremarkable. Moreover, Nagata et al. reported that MR blockade resulted in the attenuation of left ventricular hypertrophy and failure, without an antihypertensive effect, in rats with low-aldosterone hypertension. Cortisol, which manifests the same affinity for the MR as ALD, has been
described as one explanation for the activation of MR in previous reports 18, 19, 23.

The cause or effect of the prognostic impact of high cortisol remains unknown in the present study; however, combined measurements of BNP and cortisol may be useful for monitoring CHF patients (Figure 3). Further studies are needed to determine whether serial measurements of cortisol provide useful information for the management of CHF patients.

**Correlation with serum cortisol levels**

Higher cortisol levels have been reported in acute heart failure and CHF with cardiac cachexia 34, 35, but those levels in CHF patients remain unclear. Certainly, mean serum cortisol levels were almost within the normal range in this study population, but Güder et al. suggested that normal circulating cortisol concentrations may be sufficient to occupy cardiac MR 24. In the present study, serum cortisol levels were positively correlated with PCWP and NE and negatively correlated with LVEF and cardiac index (Table 3), suggesting that serum cortisol levels may reflect worse hemodynamic parameters and systemic sympathetic nerve activity in CHF because cortisol is considered a nonspecific indicator of stress.
Furthermore, in the present study, serum cortisol levels were correlated with plasma ALD levels (Table 3). The mechanism on cortisol secretion is mainly regulated by ACTH. Alternatively, ACTH is one of the mechanisms involved in ALD secretion, thus there would be a positive correlation between serum cortisol and plasma ALD in the present study. Actually, serum ACTH levels were positively correlated with plasma ALD ($r=0.379$, $p<0.0001$) and serum cortisol ($r=0.597$, $p<0.0001$) in the present study.

**Study limitation**

Several limitations should be noted in the interpretation of these results. First, the present study was performed in a single center study analyzing a small number of subjects, and only a small number of cardiac events occurred. Therefore, it might be insufficient to determine independent predictors by multivariate analyses. A larger study is warranted to confirm the prediction of cardiac events based on serum cortisol levels in CHF patients.

Second, serum cortisol levels show changes throughout the day. Although blood samples for measuring the serum cortisol levels were collected between 9:00 a.m. and 12:00 a.m. in all patients in the present study, the best time to collect samples for the
prediction of cardiac events remains unclear. Further studies are needed to clarify this problem.

Conclusions

In conclusion, serum cortisol levels were a complementary and incremental cardiac event risk predictor in combination with BNP in CHF patients and that cardiac event prediction based on cortisol levels was influenced by oxidative stress.
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Disclosures: None
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Table 1. Clinical and hemodynamic characteristics of patients with chronic heart failure

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients</th>
<th>Cardiac events(+)</th>
<th>Cardiac events (-)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=319</td>
<td>n=29</td>
<td>n=290</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.8±0.6</td>
<td>64.2±2.7</td>
<td>66±0.6</td>
<td>0.42</td>
</tr>
<tr>
<td>Gender (male / female)</td>
<td>244/75</td>
<td>25/4</td>
<td>219/71</td>
<td>0.29</td>
</tr>
<tr>
<td>BMI (kg /m²)</td>
<td>22.9±0.2</td>
<td>22.2±1.1</td>
<td>23±0.2</td>
<td>0.27</td>
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<tr>
<td>Etiology (IHD) (%)</td>
<td>185 (58)</td>
<td>12 (41.4)</td>
<td>173 (59.7)</td>
<td>0.09</td>
</tr>
<tr>
<td>NYHA (II / III / IV)</td>
<td>211/74/34</td>
<td>12/8/9</td>
<td>199/66/25</td>
<td>&lt;0.0004</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>79.8±1.5</td>
<td>79.1±3.1</td>
<td>79.9±1.6</td>
<td>0.88</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>11.2±0.4</td>
<td>17.3±1.8</td>
<td>0.6±0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cardiac index (L/min/m²)</td>
<td>2.81±0.04</td>
<td>2.53±0.13</td>
<td>2.8±0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>46.7±0.7</td>
<td>38.3±2.2</td>
<td>47.5±0.7</td>
<td>0.0002</td>
</tr>
<tr>
<td>BNP (median) (pg/mL)</td>
<td>214.2±15.4</td>
<td>534.4±105.3</td>
<td>182.1±11.9 (110.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NE (median) (pg/mL)</td>
<td>381.2±12.3</td>
<td>559.8±60.2 (529)</td>
<td>365.2±11.8 (322)</td>
<td>0.005</td>
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<tr>
<td>PRC (median) (pg/mL)</td>
<td>113.1±15.2</td>
<td>464.4±109.2 (310)</td>
<td>77.6±10.7 (23)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Angiotensin II (median) (pg/mL)</td>
<td>20.4±2.1</td>
<td>30.1±8.2 (18.5)</td>
<td>19.5±2.1 (9)</td>
<td>0.01</td>
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<tr>
<td>ALD (median) (pg/mL)</td>
<td>70.8±3.2</td>
<td>117.3±16 (98)</td>
<td>66.1±3.0 (55)</td>
<td>0.0003</td>
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<tr>
<td>Cortisol (median) (µg/dL)</td>
<td>12.8±0.3</td>
<td>16.8±1.8 (14.8)</td>
<td>12.4±0.3 (11.9)</td>
<td>0.01</td>
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<tr>
<td>ACTH (median) (pg/mL)</td>
<td>31.9±1.9</td>
<td>36.5±4.7 (32.5)</td>
<td>31.6±2.0 (23.6)</td>
<td>0.05</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.9±0.1</td>
<td>12.5±0.4</td>
<td>12.9±0.1</td>
<td>0.27</td>
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<tr>
<td>Creatinine (mg/dL)</td>
<td>1.00±0.02</td>
<td>1.15±0.1</td>
<td>0.99±0.02</td>
<td>0.009</td>
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<tr>
<td>oxLDL (unit/mL)</td>
<td>13.7±0.5</td>
<td>17.6±2.0</td>
<td>13.2±0.6</td>
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Treatments (%)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>All patients</th>
<th>Cardiac events(+)</th>
<th>Cardiac events (-)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Spironolactone</td>
<td>129 (40.4)</td>
<td>20 (69)</td>
<td>109 (37.6)</td>
<td>0.002</td>
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<td>Loop diuretics</td>
<td>134 (42)</td>
<td>21 (72.4)</td>
<td>134 (46.2)</td>
<td>0.01</td>
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<td>ACEI / ARB</td>
<td>254 (79.6)</td>
<td>24 (82.6)</td>
<td>230 (79.3)</td>
<td>0.84</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>158 (49.5)</td>
<td>18 (62.1)</td>
<td>140 (48.3)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

BMI = body mass index; IHD = Ischemic heart disease; NYHA = New York Heart Association; PCWP = pulmonary capillary wedge pressure; LVEF = left ventricular ejection fraction; BNP = brain natriuretic peptide; NE = Norepinephrine; PRC = plasma renin concentration; ALD = aldosterone; ACTH = adrenocorticotropic hormone; oxLDL = oxidized low density lipoprotein; ACEI = angiotensin converting enzyme inhibitors; ARB = angiotensin receptor blockers.
Table 2. Univariate and multivariate Cox regression analysis of risk factors for cardiac events in patients with chronic heart failure

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard Ratio</th>
<th>95% Confidence Intervals</th>
<th>p value</th>
<th>Hazard Ratio</th>
<th>95% Confidence Intervals</th>
<th>p value</th>
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</thead>
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<td>Age (years)</td>
<td>0.99</td>
<td>0.96-1.02</td>
<td>0.44</td>
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<td>Gender (male=1)</td>
<td>1.93</td>
<td>0.67-5.54</td>
<td>0.22</td>
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<td></td>
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<tr>
<td>NYHA (III/IV=1)</td>
<td>3.19</td>
<td>1.52-6.69</td>
<td>0.002</td>
<td></td>
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<tr>
<td>Mean blood pressure (&lt; 80mmHg =1)</td>
<td>3.08</td>
<td>1.43-6.64</td>
<td>0.004</td>
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<tr>
<td>LVEF (&lt; 47.5%=1)</td>
<td>3.25</td>
<td>1.39-7.61</td>
<td>0.007</td>
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<td></td>
<td></td>
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<tr>
<td>PCWP (&gt; 15mmHg=1)</td>
<td>4.04</td>
<td>1.94-8.41</td>
<td>0.0002</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cardiac index (&lt; 2.2L/min/m²=1)</td>
<td>2.1</td>
<td>0.97-4.53</td>
<td>0.05</td>
<td></td>
<td></td>
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<tr>
<td>BNP (&gt; 187pg/mL=1)</td>
<td>4.11</td>
<td>1.9-8.88</td>
<td>0.0003</td>
<td>4.4</td>
<td>1.96-9.84</td>
<td>0.0003</td>
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<tr>
<td>Norepinephrine (&gt; 378pg/mL=1)</td>
<td>3.37</td>
<td>1.56-7.25</td>
<td>0.002</td>
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<tr>
<td>PRC (&gt; 54pg/mL=1)</td>
<td>4.6</td>
<td>2.08-10.1</td>
<td>0.0002</td>
<td>3.6</td>
<td>1.58-8.21</td>
<td>0.002</td>
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<td>Angiotensin II (&gt; 12pg/mL=1)</td>
<td>4.08</td>
<td>1.8-9.22</td>
<td>0.0007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone (&gt; 89pg/mL=1)</td>
<td>4.34</td>
<td>2.07-9.1</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol (&gt; 12.5μg/dL)</td>
<td>4.71</td>
<td>1.8-12.3</td>
<td>0.002</td>
<td>3.19</td>
<td>1.16-8.74</td>
<td>0.02</td>
</tr>
<tr>
<td>ACTH (&gt; 21.8pg/mL )</td>
<td>3.65</td>
<td>1.23-10.9</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (&gt; 11.9g/dL=1)</td>
<td>1.71</td>
<td>0.82-3.56</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Creatinine (&gt; 1.25mg/dL=1)</td>
<td>2.34</td>
<td>1.06-5.13</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxLDL (&gt; 12unit/mL=1)</td>
<td>3.22</td>
<td>1.37-7.56</td>
<td>0.007</td>
<td>3.93</td>
<td>1.64-9.45</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.
Table 3. Univariate and multivariate predictors of serum cortisol levels in patients with chronic heart failure

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate correlation coefficients</th>
<th>p value</th>
<th>Multivariate β-coefficients</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yeas)</td>
<td>-0.227</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (male=1)</td>
<td>0.197</td>
<td>0.0004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NYHA (III / IV=1)</td>
<td>0.128</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>0.1</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>0.217</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac index (L/min/m²)</td>
<td>-0.01</td>
<td>0.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>-0.174</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log BNP (pg/mL)</td>
<td>0.029</td>
<td>0.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log Norepinephrine (pg/mL)</td>
<td>0.151</td>
<td>0.007</td>
<td>0.116</td>
<td>0.02</td>
</tr>
<tr>
<td>Log PRC (pg/mL)</td>
<td>0.158</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log Angiotensin II (pg/mL)</td>
<td>0.169</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log Aldosterone (pg/mL)</td>
<td>0.415</td>
<td>&lt;0.0001</td>
<td>0.162</td>
<td>0.004</td>
</tr>
<tr>
<td>Log ACTH (pg/mL)</td>
<td>0.597</td>
<td>&lt;0.0001</td>
<td>0.512</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>0.147</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.031</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxLDL (unit/mL)</td>
<td>0.023</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1. β-coefficients = standardized regression coefficients.
Figure legends

**Figure 1.** Kaplan-Meier event-free curves for CHF patients divided into 2 groups based on cut-off levels of BNP(A), NE (B), PRC (C), ALD (D) and cortisol (E). BNP = brain natriuretic peptide, NE = norepinephrine, PRC = plasma renin concentration, ALD = aldosterone.

**Figure 2.** Hazard ratio of cardiac events. Estimates were adjusted for age, sex and BMI. The square shows the hazard ratio and 95% confidence intervals for cardiac events, with patients stratified according to subgroups prespecified in the statistical analysis plan. HR = hazard ratio, CI = confidence Intervals, BNP = brain natriuretic peptide, NE = norepinephrine, PRC = plasma renin concentration, ALD = aldosterone, ACTH = adrenocorticotropic hormone, oxLDL = oxidized low density lipoprotein.

**Figure 3.** A: Kaplan-Meier event-free curves for CHF patients stratified into 4 groups based on cut-off levels of the combination of brain natriuretic peptide (BNP) and cortisol.
B: Usefulness of cardiac event prediction using a combination of plasma brain BNP levels and serum cortisol levels. Hazard ratio for comparison with the referent (ie, “cortisol low–BNP low”): for “cortisol low–BNP high”: 4.6 (p=0.05); for “cortisol high–BNP low”: 4.8 (p=0.04); for “cortisol high–BNP high”: 15.5 (p=0.0003). Interaction (-) (p=0.725).

Figure 4. A: Kaplan-Meier event-free curves for CHF patients stratified into 4 groups based on cut-off levels of the combination of serum cortisol and plasma oxidized low density lipoprotein (oxLDL), a biomarker of oxidative stress.

B: Usefulness of cardiac event prediction using a combination of serum cortisol levels and plasma oxLDL. Hazard ratio for comparison with the referent (ie, “cortisol low–oxLDL low”): for “cortisol low–oxLDL high”: 3.6 (p=0.25); for “cortisol high–oxLDL low”: 5.2 (p=0.13); for “cortisol high–oxLDL high”: 18.4 (p=0.004). Interaction (-) (p=0.976).
Figure 1

(A) Cumulative event free (%) for BNP < 187 pg/mL (n=205) vs BNP ≥ 187 pg/mL (n=114). Logrank test, p<0.0001.

(B) Cumulative event free (%) for NE < 378 pg/mL (n=201) vs NE ≥ 378 pg/mL (n=118). Logrank test, p=0.0001.

(C) Cumulative event free (%) for PRC < 54 pg/mL (n=208) vs PRC ≥ 54 pg/mL (n=111). Logrank test, p<0.0001.

(D) Cumulative event free (%) for ALD < 89 pg/mL (n=235) vs ALD ≥ 89 pg/mL (n=84). Logrank test, p<0.0001.

(E) Cumulative event free (%) for Cortisol < 12.5 µg/dL (n=159) vs Cortisol ≥ 12.5 µg/dL (n=163). Logrank test, p=0.0005.
Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP ≥ 187pg/mL</td>
<td>4.4 (1.96-9.84)</td>
</tr>
<tr>
<td>NE ≥ 378pg/mL</td>
<td>1.47 (0.51-4.2)</td>
</tr>
<tr>
<td>PRC ≥ 54pg/mL</td>
<td>3.6 (1.58-8.21)</td>
</tr>
<tr>
<td>ALD ≥ 89pg/mL</td>
<td>2.18 (0.78-6.06)</td>
</tr>
<tr>
<td>Cortisol ≥ 12.5μg/dL</td>
<td>3.19 (1.16-8.72)</td>
</tr>
<tr>
<td>ACTH ≥ 21.8pg/mL</td>
<td>1.42 (0.4-4.96)</td>
</tr>
<tr>
<td>oxLDL ≥ 12unit/mL</td>
<td>3.93 (1.64-9.45)</td>
</tr>
</tbody>
</table>
Figure 3

Logrank test, p<0.0001

Hazard ratio

BNP > 187pg/mL, Cortisol > 12.5g/dL (n=68)
BNP < 187pg/mL, Cortisol < 12.5g/dL (n=110)
BNP < 187pg/mL, Cortisol > 12.5g/dL (n=95)
BNP ≥ 187pg/mL, Cortisol ≥ 12.5g/dL (n=68)
Figure 4

- Logrank test, p<0.0001

- Hazard ratio
  - Cortisol < 12.5 µg/dL, oxLDL < 12 unit/mL (n=81)
  - Cortisol < 12.5 µg/dL, oxLDL ≥ 12 unit/mL (n=75)
  - Cortisol ≥ 12.5 µg/dL, oxLDL < 12 unit/mL (n=83)
  - Cortisol ≥ 12.5 µg/dL, oxLDL ≥ 12 unit/mL (n=80)

- Cumulative event free (%)
  - Months: 0, 10, 20, 30, 40, 50, 60, 70

- Hazard ratio
  - P=0.008
  - P=0.004

- oxLDL ≥ 12 unit/mL
  - 18.4

- oxLDL < 12 unit/mL
  - 5.2

- Cortisol ≥ 12.5 µg/dL
  - 3.6

- Cortisol < 12.5 µg/dL
  - 1
Serum Cortisol as a Useful Predictor of Cardiac Events in Patients with Chronic Heart Failure: the Impact of Oxidative Stress
Masayuki Yamaji, Takayoshi Tsutamoto, Chiho Kawahara, Keizo Nishiyama, Takashi Yamamoto, Masanori Fujii and Minoru Horie

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