Cystatin C as a Predictor of Mortality and Cardiovascular Morbidity After Cardiac Resynchronization Therapy

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**Background:** Cardiac resynchronization therapy (CRT) has been reported to improve symptoms and cardiac performance in patients with severe heart failure (HF), but CRT recipients with advanced HF do not always experience improved mortality rates. Cystatin C has recently been involved in HF, but the association of serum cystatin C level with adverse events and long-term prognosis after CRT is unknown. This study investigated whether cystatin C level can predict mortality and cardiovascular events after CRT.

**Methods and Results:** A total of 117 consecutive patients receiving a CRT device for the treatment of advanced HF were assessed according to cystatin C level and long-term outcome after implantation of the device. Over a median follow-up of 3.2 years, 34 patients (29.1%) died and 59 patients (50.4%) developed cardiovascular events. Kaplan-Meier survival analysis indicated that elevated cystatin C level was significantly associated with higher all-cause mortality and prevalence of cardiovascular events, including hospitalization for progressive HF. After multivariate Cox regression analysis, serum cystatin C level and QRS duration, but not conventional echocardiographic parameters, were found to independently predict all-cause death or cardiovascular events. Of importance, only cystatin C level was an independent predictor of all-cause mortality after CRT.

**Conclusions:** Cystatin C level independently predicts cardiac mortality or morbidity in patients receiving CRT. The assessment of cystatin C level could provide valuable information about long-term prognosis after CRT. (Circ J 2013; 77: 2751–2756)

**Key Words:** Biomarker; Cardiac resynchronization therapy; Cystatin C

Cardiac resynchronization therapy (CRT) has been established as a therapeutic option in patients with advanced heart failure (HF). Accumulating evidence has shown that CRT decreases mortality and hospital admission rates, but approximately one-third of patients do not respond to CRT. Based on current knowledge of the involved mechanisms, patients with beneficial CRT outcomes have consisted of those suffering impairment of electromechanical ventricular dyssynchrony leading to reverse remodeling. It is difficult, however, to identify which patients will have the best outcome after CRT from pre-implant assessments because echocardiographic measurements of dyssynchrony have completely failed in patient selection for CRT, whereas restoring dyssynchrony is one of the important factors for the prognosis of CRT. Therefore, it is clinically valuable to identify pre-implant characteristics that predict good outcome after CRT.

Approximate two-thirds of patients hospitalized with HF have renal failure. The presence of renal insufficiency in patients receiving CRT has been associated with an increased risk of mortality and cardiovascular events. Serum creatinine, however, is the usual clinical tool for measuring renal function, is not useful for the detection of moderate renal dysfunction because it is affected by various factors unrelated to renal function, such as age, sex, race, and lean muscle mass. In contrast, severe HF is associated with age, gender and muscle atrophy by cardiac cachexia. Consequently, in patients receiving CRT, the evaluation of renal function using creatinine is likely problematic.

Cystatin C, a cysteine protease inhibitor produced at a constant rate by nearly all human cells, is freely filtered by the renal glomerulus and excreted into the bloodstream. This protein has a low molecular weight and does not form a complex with...
other secreted proteins in the blood. Therefore the serum level of cystatin C does not depend on patient size, has a strong negative correlation with glomerular filtration rate (GFR) and is clearly superior to serum creatinine as a marker of GFR. Given its reported superiority over creatinine as a proxy for GFR, we hypothesized that cystatin C could predict the risk of illness and death after CRT. In the present study, we investigated whether this secreted protein could predict long-term outcome after CRT.

### Methods

#### Subjects
We assessed 119 consecutive patients with HF who underwent CRT at Nagoya University Hospital between September 2003 and December 2011. The patients were selected according to the following established selection criteria for CRT of the Cardiac REsynchronization in HF (CARE-HF): (1) severe HF despite optimized medical therapy; (2) left ventricular (LV) systolic dysfunction with an LV ejection fraction (LVEF) <35%; and (3) QRS duration >120 ms. Additionally, patients with a QRS interval 120–149 ms were required to meet 2 of 3 additional criteria for dyssynchrony (aortic pre-ejection delay >140 ms, interventricular mechanical delay >40 ms, delayed activation of the posterolateral LV wall). Two patients with hemodialysis were excluded from the study. All subjects provided written informed consent.

#### Laboratory Measurement
Fasting blood samples were obtained from every patient. After each patient rested for 10 min in the supine position, their vital signs were recorded and a 35-ml blood sample was collected from the antecubital vein. Serum cystatin C level was measured by SRL (Tokyo, Japan), a commercial clinical testing laboratory.

#### Echocardiography
Two-dimensional echocardiography was performed by 2 experienced sonographers using a Vivid 7 Dimension/Pro System (GE Healthcare, UK) before the CRT device was implanted. The images were recorded, using a 3.5-MHz transducer, in cineloop format and were digitally stored for offline analysis. LV end-diastolic diameter (LVEDD) and LV end-diastolic volume (LVEDV) were measured as recommended by the American Society of Echocardiography. LVEF was calculated using a modified Simpson’s rule. Septal-to-posterior wall motion delay (SPWMD) and LV pre-ejection period (LV-PEP) were assessed as dyssynchrony parameters.

#### Follow-up and Assessment of Cardiac Events
All patients underwent regular follow-up (typically every 2 months) via outpatient clinical visits or telephone interview. Cardiovascular events including hospitalizations for exacerbation of HF were adjudicated by cardiologists from Nagoya University Hospital. Causes of death were ascertained by reviewing the clinical records.

#### Statistical Analysis
All data are expressed as mean ± SD. Univariate and multivariate Cox regression analysis was performed to control for potentially confounding echocardiographic, demographic and clini-
Cystatin C Level After CRT

Figure. Kaplan-Meier estimates of the time to various clinical endpoints: (A) all-cause death; (B) cardiovascular events. Patients were stratified according to serum cystatin C level: T1, ≤1.14 mg/L; T2, 1.14–1.54 mg/L; T3, >1.54 mg/L.

Results

Baseline Characteristics

Baseline characteristics are listed in Table 1. Cystatin C level ranged from 0.72 to 5.62 mg/L (median, 1.31 mg/L). The range of cystatin C level was higher than the tertiles of 117 patients by tertile as follows: tertile 1, ≤1.14 mg/L; tertile 2, 1.14–1.54 mg/L; and tertile 3 >1.54 mg/L. CRT recipients were 67.0±11.0 years of age and 65.8% of the men were predominantly classified as New York Heart Association (NYHA) functional class III or IV with QRS prolongation (165±29 ms). The etiology of HF in 22.2% of the patients was ischemic heart disease. Participants with high cystatin C level were older and more likely to have ischemic etiology than those with normal/low cystatin C level. We found no differences, however, in lipid profile as an ischemic risk factor between cystatin C tertiles. Eight patients (6.8%) had valvular heart disease that required surgical repair. Eleven patients (9.4%) previously had pacers or defibrillator devices implanted. Serum creatinine, brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) levels were higher in patients with elevated cystatin C prior to CRT device implantation. Additionally, cystatin C level was significantly correlated with creatinine level (R²=0.741) and estimated GFR (eGFR; R²=821). Patients had severe LV dysfunction (mean LVEF, 27.0±7.6%), with extensive dilatation (LVEDD, 69.0±9.7 mm, LVEDV 228±91 ml). There were no differences in conventional echocardiographic parameters including dysynchrony parameters between cystatin C tertiles.

Clinical Outcome During Follow-up

Mean follow-up duration was 3.21±2.11 years. There were 34 deaths (29%). Causes of death included non-cardiac-related death in 3 patients (cancer, n=2; traumatic accident, n=1). A total of 59 patients (50.4%) were hospitalized for cardiovascular events, including exacerbation of HF after CRT device implantation.

Prediction of Outcome After CRT

We first performed Kaplan-Meier survival analysis in the 3 groups according to serum cystatin C level. It was found that patients with elevated cystatin C had significantly higher all-cause mortality (Figure A) than the others. Subsequently, we performed univariate Cox regression analysis to examine the correlation between conventional risk factors including cystatin C and long-term prognosis (all-cause mortality) after CRT. There were statistically significant differences in serum cystatin C level, ischemic heart disease, and QRS duration before CRT device implantation (Table 2), but we found no significant differences in serum BNP and ANP levels, age, echocardiographic parameters (LVEDD, LVEDV, LVEF, SPWMD, and LV-PEP), and upgrading of pre-existing pacing devices (Table 2). Of note, only baseline cystatin C level (P<0.01) before CRT device implantation was an independent predictor of all-cause mortality in multivariate Cox regression modeling, after controlling for ischemic cardiomyopathy etiology and eGFR width (Table 2).

Kaplan-Meier survival analysis indicated that high cystatin C tertile was significantly associated with a higher prevalence of combined endpoints (death or cardiovascular events hospitalization, including HF deterioration) compared to the other tertiles (Figure B). Moreover, serum cystatin C level, ischemic
higher mortality rate or cardiovascular events hospitalization than those in the other tertiles, on Kaplan-Meier survival analysis (Figure S1). Additionally, creatinine-based eGFR is independently associated with both cardiac mortality and morbidity even if eGFR, instead of cystatin C, is included in Cox models (Tables S1, S2).

**Discussion**

This study has shown for the first time that serum cystatin C level prior to CRT device implantation independently predicts mortality and morbidity in patients receiving CRT. Moreover, the association of cystatin C with mortality is superior to that of serum BNP level. Consequently, cystatin C could potentially provide a risk stratification of CRT recipients.

Cardio-renal syndrome, renal dysfunction associated with chronic HF, confers a worse prognosis independent of LVEF. Unsurprisingly, severe chronic kidney disease is associated with worse outcome after device therapy. Serum creatinine or creatinine-based equations used to estimate GFR predict good long-term clinical outcome after CRT, while their precision when applied to patients receiving CRT is unclear because baseline cystatin C level and QRS duration before CRT device implantation were independent predictors of death or cardiovascular event-related hospitalization (Table 3).

Finally, we examined the superiority of cystatin C to creatinine and creatinine-based eGFR, but we could not exclude co-linearity in Cox regression analysis because the correlation between the three measures was very high. Therefore we investigated combined ROC curves for cystatin C, eGFR, and creatinine for all-cause death or cardiovascular events. On ROC analysis for cystatin C, eGFR, and creatinine, AUC was 0.731, 0.724, and 0.678 for mortality and 0.743, 0.738 and 0.698 for death or cardiovascular events, respectively. The largest AUC was obtained with cystatin C, which is likely superior to creatinine but equal to eGFR, and therefore we were able to show that patients in the high tertile of eGFR significantly had a higher mortality rate or cardiovascular events hospitalization than those in the other tertiles, on Kaplan-Meier survival analysis (Figure S1). Additionally, creatinine-based eGFR is independently associated with both cardiac mortality and morbidity even if eGFR, instead of cystatin C, is included in Cox models (Tables S1,S2).

**Table 2. Predictors of All-Cause Death**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Univariate</th>
<th>Multivariate</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HR 95% CI</td>
<td>P-value</td>
</tr>
<tr>
<td>Cystatin C (1-mg/L increment)</td>
<td>1.748</td>
<td>1.300–2.351</td>
</tr>
<tr>
<td>Age</td>
<td>1.010</td>
<td>0.976–1.045</td>
</tr>
<tr>
<td>Ischemic etiology</td>
<td>2.097</td>
<td>1.035–4.248</td>
</tr>
<tr>
<td>Valvular heart disease</td>
<td>1.171</td>
<td>0.279–4.926</td>
</tr>
<tr>
<td>Upgrading of existing device</td>
<td>0.043</td>
<td>0.000–9.772</td>
</tr>
<tr>
<td>Log BNP</td>
<td>1.560</td>
<td>0.709–3.430</td>
</tr>
<tr>
<td>Log ANP</td>
<td>1.211</td>
<td>0.491–2.984</td>
</tr>
<tr>
<td>LVEDD</td>
<td>0.991</td>
<td>0.956–1.027</td>
</tr>
<tr>
<td>LVEDV</td>
<td>0.997</td>
<td>0.954–1.042</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.995</td>
<td>0.995–1.003</td>
</tr>
<tr>
<td>SPWMD (ms)</td>
<td>0.996</td>
<td>0.991–1.001</td>
</tr>
<tr>
<td>LV-PEP (ms)</td>
<td>1.053</td>
<td>0.921–1.203</td>
</tr>
<tr>
<td>QRS width (ms)</td>
<td>0.985</td>
<td>0.973–0.997</td>
</tr>
</tbody>
</table>

CI, confidence interval; HR, hazard ratio. Other abbreviations as in Table 1.

**Table 3. Predictors of Combined Primary Endpoints†**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 95% CI</td>
<td>P-value</td>
</tr>
<tr>
<td>Cystatin C (1-mg/L increment)</td>
<td>1.896</td>
<td>1.475–2.409</td>
</tr>
<tr>
<td>Age</td>
<td>0.999</td>
<td>0.975–1.024</td>
</tr>
<tr>
<td>Ischemic etiology</td>
<td>1.819</td>
<td>1.037–3.190</td>
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<tr>
<td>Valvular heart disease</td>
<td>0.962</td>
<td>0.298–3.105</td>
</tr>
<tr>
<td>Upgrading of existing device</td>
<td>0.162</td>
<td>0.022–1.172</td>
</tr>
<tr>
<td>Log BNP</td>
<td>2.196</td>
<td>1.191–4.050</td>
</tr>
<tr>
<td>Log ANP</td>
<td>1.527</td>
<td>0.768–3.038</td>
</tr>
<tr>
<td>LVEDD</td>
<td>1.012</td>
<td>0.986–1.039</td>
</tr>
<tr>
<td>LVEDV</td>
<td>1.001</td>
<td>0.998–1.004</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.995</td>
<td>0.962–1.030</td>
</tr>
<tr>
<td>SPWMD (ms)</td>
<td>0.998</td>
<td>0.994–1.001</td>
</tr>
<tr>
<td>LV-PEP (ms)</td>
<td>1.008</td>
<td>0.990–1.026</td>
</tr>
<tr>
<td>QRS width (ms)</td>
<td>0.984</td>
<td>0.975–0.993</td>
</tr>
</tbody>
</table>

†Death or cardiovascular events. Abbreviations as in Table 1,2.
cause the CRT recipients in the studies were relatively old and lean.\(^{35,36}\) The utility of cystatin C, however, was almost equal to that of eGFR in the present study. We previously demonstrated baseline contractile function, rather than improvement of dysynchrony, predicted mortality and cardiovascular events.\(^{37}\) These findings suggest that CRT recipients are more likely to die if they have bad kidneys and bad hearts. Thus, we showed that serum cystatin C level at baseline independently predicted all-cause mortality or cardiovascular event-related hospitalization in patients receiving CRT. These data suggest that measurement of serum cystatin C level before CRT device implantation could provide useful mortality and morbidity information for patients undergoing CRT.

Observational studies have shown that renal failure independently predicted all-cause mortality among patients with heart failure before and after CRT.\(^{30,22,23,34}\) The reason for renal insufficiency being such a sensitive index for mortality response to CRT is thought to involve the manner in which impaired renal function increases volume expansion secondary to sodium retention. To the best of our knowledge, many studies have evaluated the clinical impact of cystatin C level on mortality in patients receiving CRT. These data suggest that measurement of serum cystatin C level before CRT device implantation could provide useful mortality and morbidity information for patients undergoing CRT.

The limitations of the present study, however, are similar to those of any single-center observational study. Second, we did not show the superiority of cystatin C to creatinine based on renal dysfunction in this study. Finally, we had no data on serial measurements of cystatin C. When we used ROC to analyze the serial measurement of eGFR before and at 1 month, 3 months, and 6 months after device implantation with regard to mortality and cardiovascular events in patients who survived >6 months, the eGFR before and at 6 months after device implantation had a larger AUC compared with 1 and 3 months after. Thus eGFR might be superior to cystatin C because eGFR is serially measured in real-world clinical practice. A future large clinical trial is required to confirm the utility of serial measurements of cystatin C.

Taken together, the present observations suggest that cystatin C level predicts cardiac mortality and morbidity in patients receiving CRT. Measurement of this serum protein could provide useful information about long-term prognosis after CRT. More careful assessments should be considered for attentive follow-up, whereas the assessment of cystatin C is independent of the option of whether to undergo CRT or not.

**Conclusions**

In severe HF patients receiving CRT, cystatin C had a strong predictive value for mortality and cardiovascular events after device implantation.

**Acknowledgments**

We gratefully acknowledge the assistance of Rieko Ito, Hatsumi Inaba and Kaori Kato. This work was supported by Japan Foundation for Applied Enzymology and Grant-in-Aid for Young Scientists B23790844 to M.S.

**Disclosures**

Conflicts of Interest: None.

**References**


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**Supplementary Files**

**Supplementary File 1**

**Figure S1.** Kaplan-Meier Estimates of the time to various clinical endpoints.

**Table S1.** Predictors of all-cause death

**Table S2.** Predictors of combined primary endpoints

Please find supplementary file(s); http://dx.doi.org/10.1253/circj.CJ-13-0179