Cystatin C levels are associated with the prognosis of systolic heart failure patients

Les concentrations de cystatine C sont un facteur pronostique dans l’insuffisance cardiaque

Changlu Gao\textsuperscript{a,1}, Lihua Zhong\textsuperscript{b,1}, Yanhui Gao\textsuperscript{b}, Xueqi Li\textsuperscript{b}, Mingyu Zhang\textsuperscript{b}, Shipeng Wei\textsuperscript{b,∗}

\textsuperscript{a} Department of Surgical Oncology, the 4th Clinical Hospital of Harbin Medical University, Harbin, China
\textsuperscript{b} Department of Cardiology, The Fourth Affiliated Hospital of Harbin Medical University, 37 Yiyuan Str., Nangang District, Harbin 150001, China

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KEYWORDS
Heart failure; Homocysteine; High-sensitivity C-reactive protein; Cystatin C

Summary
Background. — Cystatin C, which has long been regarded as a biomarker that indicates kidney functions, has recently been recognized as an inflammatory marker in the human body.
Aim. — To elucidate how cystatin C is related to the prognosis of systolic heart failure patients.
Methods. — Patients with systolic heart failure who were admitted to the fourth affiliated hospital of Harbin Medical University between January and April 2008 were enrolled in this study. Serum homocysteine, high-sensitivity C-reactive protein (hs-CRP) and cystatin C levels were determined and all the patients received an average of 2 years of follow-up for occurrence of death, heart transplantation or readmission with worsening heart failure.
Results. — Of 138 patients enrolled, those who experienced adverse outcomes (e.g. cardiac death, heart transplantation or progressive heart failure) \((n = 21)\) had considerably higher mean levels of serum homocysteine \((28.6 \pm 13.4 \text{ vs } 14.4 \pm 6.3 \text{ mg/L}; \ p < 0.01)\), hs-CRP \((17.5 \pm 14.1 \text{ vs } 6.4 \pm 7.7 \mu \text{mol/L}; \ p < 0.01)\) and cystatin C \((1.63 \pm 0.81 \text{ vs } 0.91 \pm 0.27 \text{ mg/L}; \ p < 0.01)\) than those without adverse outcomes \((n = 117)\). Furthermore, the Cox proportional hazards model demonstrated that serum homocysteine, hs-CRP and cystatin C are all independent predictors of adverse outcomes.
Conclusions. — Cystatin C, together with hs-CRP and homocysteine, is an independent risk factor that is important in the prognosis of patients with systolic heart failure.

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\textsuperscript{∗} Corresponding author. Fax: +86 451 8257 6977.
E-mail address: shipengwei@yahoo.com (S. Wei).
\textsuperscript{1} Dr. Gao and Zhong contributed equally as first authors.

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Background

Heart failure is the end stage of heart disease. It is usually irreversible, and is characterized by high mortality. The methods used to treat or manage heart failure (such as heart transplantation and stem-cell transplantation) have only limited effects [1], indicating that heart failure does not merely cause damage to the heart, but that it is a systemic disease. For this reason, the above-mentioned methods cannot represent a complete cure. In the past two decades, scholars have realized that oxidative stress is one of the most important causes of heart failure because the biomarkers of oxidative stress are extremely high in patients suffering from heart failure compared to healthy individuals [2—8]. Furthermore, treatments focused on oxidative stress can relieve symptoms and increase the cardiac function in both heart failure patients and animal models [9—12]. The levels of these biomarkers are thought to be highly valuable for the evaluation of the severity of heart failure and the effectiveness of treatments. Such biomarkers include thiobarbituric acid-reactive substances and 8-isoprostaglandin F2alpha [2,13].

Cystatin C is one of the most frequently studied biomarkers that indicate the level of oxidative stress in the human body; and is closely related to heart disease. Lee et al. found that patients with coronary artery disease exhibit increased serum cystatin C levels [14]. The same observation is evident among patients with hypertension [15]. However, the manner by which cystatin C is related to systolic heart failure and its prognosis remains unknown. We hypothesized that patients experiencing systolic heart failure would have higher cystatin C levels, and that cystatin C exhibits an inverse relationship with the prognosis of systolic heart failure. We therefore measured cystatin C levels in patients with systolic heart failure. We also followed these patients for an average of 2 years to verify our hypothesis.

Methods

Study population

Consecutive patients with either chronic or new-onset systolic heart failure (effort dyspnoea or echocardiographic findings of systolic dysfunction) who were admitted to our hospital between January and April 2008 were screened for inclusion into this prospective study. The significance of heart failure was determined by both echocardiography and NYHA classification. Only patients who successfully underwent serum hs-CRP, homocysteine and cystatin C measurements besides regular examinations (e.g. blood test, echocardiogram, electrocardiogram, etc.) qualified for inclusion. Exclusion criteria included significant valvular heart disease, active myocarditis, known congenital heart disease, recent acute coronary syndrome (within 6 months), pending revascularization, uncontrolled hypertension, and/or significant endocrine, hepatic, renal or inflammatory...
Cystatin C and heart failure

Left ventricular ejection fraction

The Vivid 7 color Doppler ultrasonic (GE Healthcare, Shanghai, China) was used for the evaluation of cardiac function. The left ventricular endocardial borders were recorded from the projected left ventriculogram in end systole and diastole. Ejection fractions of patients were confirmed by echocardiographic assessment.

Laboratory tests

After an overnight fast (≥12 h), blood samples were obtained from all patients after bed rest for 15 min in a quiet room in the morning hours before enrolment as described previously [16]. Homocysteine, hs-CRP, cystatin C, total serum bilirubin, total cholesterol, triglycerides, LDL and HDL were measured and recorded for analysis.

Long-term follow-up

All patients underwent long-term follow-up. Periodical physical examinations were performed every 3 months either at home or in the hospital. Blood tests were performed at the same time. To achieve a maximum follow-up duration of 3 years, vital signs (blood pressure and heart rate) were determined for all enrolled patients as of April 2008. During long-term follow-up, the adverse outcomes defined as primary endpoints were: (1) death from cardiac causes (defined as sudden cardiac death or death resulting from progressive pump failure); (2) development or progression of heart failure that required hospitalization for an intravenous treatment of heart failure within the first 3 days after admission; or (3) heart transplantation. For patients experiencing >1 adverse outcome, only the first one was considered. All information regarding potential adverse outcomes was acquired by review of source data, including hospital record forms, death certificates and other original documents. Other vascular events, such as stroke, nonfatal myocardial infarction or coronary revascularization, were also assessed, but not included into the outcome analysis. Medical therapy, including ACE inhibitors, ARBs, beta blockers, digoxin and statins did not differ significantly among the groups with and without adverse outcomes.

Statistics analysis

SPSS 17.0 software was used to analyze the data. Results are reported as group means ± standard deviation (SD). A one-way ANOVA was used to determine differences among the group means. Differences at \( P < 0.05 \) were considered significant. Cumulative adverse outcome rates were estimated by Kaplan-Meier survival curves and compared by the log-rank test. For survival analysis, patients were split into two groups, based on the median values, for each of serum homocysteine, hs-CRP and cystatin C. Cox proportional hazards regression analysis was used to analyze follow-up data. Among all the variables tested, only those with statistical significance set at \( P < 0.05 \) at univariate analysis were included in a multivariable Cox regression model to determine independent predictors of adverse outcomes.

Results

Patient characteristics

Of 168 patients screened, 138 met the inclusion criteria and had complete clinical data. The mean follow-up time was 2 years (range 0.3–3 years). During follow-up, 32 adverse outcomes in 21 patients were documented: 10 patients died of cardiac causes (seven sudden death and three refractory heart failure); two patients received heart transplantation and survived until the end of follow-up; and nine had exacerbation of heart failure as observed in the worsening of NYHA functional class and required re-hospitalization and intravenous treatment for heart failure. Eleven patients suffered from one adverse outcome, nine patients from two adverse outcomes, one from three adverse outcomes. No patients died of non-cardiac causes.

Patient characteristics and clinical outcomes are shown in Table 1. The proportion of patients receiving pharmacological agents such as ACE inhibitors, ARBs, beta blockers, digoxin and statins did not differ significantly among the groups with and without adverse outcomes.

Differences in serum homocysteine, hs-CRP and cystatin C levels between the two groups

The serum levels of homocysteine, hs-CRP, and CysC were determined. As shown in Table 1, patients experiencing adverse outcomes had a significantly higher level of homocysteine than did those without adverse outcomes (28.6 ± 13.4 vs 14.4 ± 6.3; \( P < 0.001 \)). Similar results were seen for hs-CRP levels (17.5 ± 14.1 vs 6.4 ± 7.7; \( P < 0.001 \)) and cystatin C levels (1.63 ± 0.81 vs 0.91 ± 0.27 mg/L; \( P < 0.001 \)) (Table 1).

Cystatin C was an independent predictor in patients with heart failure

Further analysis was carried out to determine how cystatin C and other factors are associated with the prognosis of heart failure patients. In this study, we also found that homocysteine was another independent risk factor in the prognosis of systolic heart failure patients. In the univariate Cox regression analysis (Table 2), male gender, history of hypertension, lower triglycerides, and higher serum creatinine, homocysteine, hs-CRP and cystatin C levels were all significantly related to poor prognosis among patients with systolic heart failure. In the subsequent multivariate analysis, only triglycerides, homocysteine, hs-CRP and cystatin C levels showed a significant relationship with prognosis (Table 3).

Patients with higher cystatin C levels had a higher rate of adverse outcomes

When comparing adverse outcomes among patients with low versus high biomarker levels, the cutoff points were chosen as the medians of the entire spectrum (0.9 mg/L for cystatin C; 4.2 μmol/L for hs-CRP and 14 mg/L for homocysteine)
As shown in Fig. 1A, patients with higher cystatin C levels had a significantly higher adverse outcome rate compared with those exhibiting lower levels of cystatin C ($P < 0.0001$). Similar results were observed in the analysis of the rate of adverse outcomes based on serum hs-CRP and homocysteine levels (Fig. 1B and C).

## Discussion

Heart failure has been a long-standing issue encountered in clinical work, and it is the end stage of heart disease. Currently, treatment methods are gradually shifting focus from mechanically or medically improving the activities of the heart to eliminating the basic causes of heart failure, on the basis of a better understanding of the underlying mechanisms [17]. Oxidative stress occurs throughout the progression of heart failure. Thus, certain factors have been associated with prognosis. Uric acid, NT-ProBNP, creatinine, hs-CRP and cystatin C have all been shown to be predictive of heart failure [18–21]. Cystatin C has also been observed at increased levels during heart failure, and serves as a highly significant indicator of the outcome of heart failure [21]. However, these studies are rare and the follow-up periods are short. Furthermore, the study populations are limited. Hence, we enrolled systolic heart failure patients with a wider spectrum to conduct this prospective study. Our intention was to carry out long-term follow-up to evaluate whether cystatin C is associated with the prognosis of these patients.

In the present study, two methods for evaluating the function of the heart were adopted: the objective measurement of ejection fraction and subjective assessment of heart function by NYHA classification. However, both NYHA and ejection fraction were unrelated to the adverse outcomes observed in the patients with heart failure. That is, although some patients had lower NYHA classifications and better ejection fractions, they continued to suffer from adverse outcomes. The factors that determined the prognosis were triglycerides, serum homocysteine, cystatin C and hs-CRP. hs-CRP is a sensitive risk factor of inflammatory diseases and has been proven to be related to heart disease [22,23]. Other factors, such as homocysteine and cystatin C, have been shown in recent years to have the same effect for...
evaluating the severity of some heart diseases [8,20]. For the prognosis of heart failure, however, reports are rare. In the current investigation, hs-CRP was found to be related to the prognosis of heart failure patients. Serum homocysteine also showed a strong relationship with prognosis, and was significantly higher among patients experiencing adverse outcomes during follow-up. As an essential amino acid, homocysteine is derived from the conversion of methionine to cysteine. Many enzymes are involved in several reactions until methionine is eventually converted into cysteine and vice versa. Folic acid and other B vitamins are important co-factors; thus, serum homocysteine levels are affected by diet. Measuring the influence of these vitamins would have enhanced the reliability of the results of this study.

Surprisingly, cystatin C showed a considerably stronger relationship with prognosis, indicating that it is a strong predictor of systolic heart failure. To the best of our knowledge, such a finding has not been reported thus far in the literature. Cystatin C, which is a more sensitive marker of renal function than creatinine, has long been used as a factor in the evaluation of renal function [24]. Thereafter, it has been shown to be associated with the severity of coronary artery disease and heart failure [20,25]. To the best of our knowledge, the present study is the first to disclose another function of cystatin C. Lipoproteins are associated with heart diseases and statin therapy is expected to reduce the adverse outcomes of heart diseases, although the results regarding its application in patients with established, and particularly, advanced heart failure remain controversial [26]. For years, high low-density lipoprotein has constantly been in the spotlight, whereas low HDL and high triglyceride content are often neglected [27]. Some researchers have reported that as a component of lipoproteins, triglyceride is related to the prognosis of patients with heart failure, but focus on this ester has not been as extensive [28]. In the current work, triglyceride was found to be a predictor in the prognosis of systolic heart failure patients. It showed an inverse relationship with adverse outcomes among patients with systolic heart failure, a result that differs from that reported in literature [28]. By looking into the data on lipoproteins, we found that lipoprotein levels in patients experiencing adverse outcomes were lower than those not suffering from adverse outcomes. Because lipoproteins are also sensitive to diet, we attributed this result to the poor diet of the patients.

Bilirubin is another factor that we assumed correlated with the prognosis of patients with systolic heart failure. A significant difference in total bilirubin between patients with and without adverse outcomes was observed (23.9 ± 14.1 vs 17.2 ± 8.6 μmol/L; P = 0.002), but in further univariate analysis, this relationship was found not to be significant. On the basis of our clinical experience, patients with severe heart failure (NYHA classifications III and IV) often have higher serum bilirubin levels than patients with mild to moderate heart failure (20.9 ± 12.1 vs 15.3 ± 5.7 μmol/L; P < 0.001). Thus, we chose serum bilirubin as a potential candidate in the prognosis of systolic heart failure.

<table>
<thead>
<tr>
<th>Table 2 Cox univariate regression analysis.</th>
<th>HR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Older age</td>
<td>2.74 (2.56–2.95)</td>
<td>0.805</td>
</tr>
<tr>
<td>Male sex</td>
<td>3.85 (3.49–7.59)</td>
<td>0.022</td>
</tr>
<tr>
<td>Lower LVEF</td>
<td>2.48 (2.27–2.78)</td>
<td>0.119</td>
</tr>
<tr>
<td>Higher NYHA score</td>
<td>1.99 (1.11–7.79)</td>
<td>0.703</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>3.88 (2.80–6.42)</td>
<td>0.043</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.94 (0.86–1.10)</td>
<td>0.299</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>2.32 (1.21–3.67)</td>
<td>0.818</td>
</tr>
<tr>
<td>Higher creatinine</td>
<td>2.67 (2.63–2.71)</td>
<td>0.027</td>
</tr>
<tr>
<td>Higher uric acid</td>
<td>2.72 (2.70–2.74)</td>
<td>0.859</td>
</tr>
<tr>
<td>Lower total cholesterol</td>
<td>1.65 (1.51–1.98)</td>
<td>0.178</td>
</tr>
<tr>
<td>Lower triglycerides</td>
<td>1.73 (1.42–2.37)</td>
<td>0.009</td>
</tr>
<tr>
<td>Lower HDL</td>
<td>1.80 (1.00–6.77)</td>
<td>0.339</td>
</tr>
<tr>
<td>Lower LDL</td>
<td>1.04 (1.00–15.91)</td>
<td>0.133</td>
</tr>
<tr>
<td>Higher total bilirubin</td>
<td>2.75 (2.61–2.91)</td>
<td>0.688</td>
</tr>
<tr>
<td>Higher BNP</td>
<td>1.12 (1.05–1.66)</td>
<td>0.110</td>
</tr>
<tr>
<td>Higher homocystine</td>
<td>3.03 (2.79–3.30)</td>
<td>0.039</td>
</tr>
<tr>
<td>Higher hs-CRP</td>
<td>2.88 (2.72–3.06)</td>
<td>0.049</td>
</tr>
<tr>
<td>Higher cystatin C</td>
<td>3.58 (2.61–4.82)</td>
<td>0.033</td>
</tr>
</tbody>
</table>

BNP: B natriuretic peptide; CI: confidence interval; HDL: high-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein; HR: hazard ratio; LDL: low-density lipoprotein; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association.

<table>
<thead>
<tr>
<th>Table 3 Cox multivariable analysis.</th>
<th>HR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>2.02 (1.71–2.51)</td>
<td>0.010</td>
</tr>
<tr>
<td>Cysteine C</td>
<td>7.10 (3.36–23.75)</td>
<td>0.006</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>3.02 (2.88–3.17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>2.93 (2.82–3.03)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CI: confidence interval; hs-CRP: high-sensitivity C-reactive protein; HR: hazard ratio.
failure patients. Given that the sample size is small, further studies including more patients would be necessary to confirm this finding. The same trend was also observed in the evaluation of age, uric acid and creatine. Although significant differences were exhibited between groups, further analysis ruled out these factors.

As inflammatory related diseases, hypertension and ischaemic heart disease accounted for a considerable amount of patients in this study, 59 and 43%, respectively. Although hypertension was significantly predictive of adverse outcomes in the univariate analysis, it was ruled out in the following multivariable analysis. Since the sample size was not large enough for this result to reach significance on multivariable analysis, further studies with larger populations would be needed to clarify this matter.

Conclusions

More adverse outcomes were observed among the systolic heart failure patients with high cystatin C levels. High serum hs-CRP and homocysteine levels were also related to the outcomes of the patients. All of these were independent risk factors in the prognosis of the patients with systolic heart failure. These results are expected to provide clinical importance in the differentiation of systolic heart failure patients at higher risk of cardiac death, heart transplantation or progression of heart failure.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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