Risk Stratification of Chronic Heart Failure Patients by Multiple Biomarkers

— Implications of BNP, H-FABP, and PTX3 —

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**Background**

B-type natriuretic peptide (BNP), heart-type fatty acid-binding protein (H-FABP), and pentraxin 3 (PTX3) each predict adverse cardiac events in chronic heart failure (CHF) patients. For prognostic evaluation from different aspects, the utility of combined measurement of the 3 biomarkers in patients with CHF was examined in the present study.

**Methods and Results**

Levels of BNP (associated with left ventricular dysfunction, positive if >200 pg/ml), H-FABP (marker of myocardial damage, positive if >4.1 ng/ml), and PTX3 (marker of inflammation, positive if >4.0 ng/ml) were measured in 164 consecutive CHF patients, and patients were prospectively followed with endpoints of cardiac death or rehospitalization. When patients were categorized on the basis of the number of elevated biomarkers, patients with 1, 2, and 3 elevated biomarkers had a 5.4-fold (not significant), 11.2-old (p<0.05), and 34.6-fold increase (p<0.01), respectively, in the risk of adverse cardiac events compared with those without elevated biomarkers. Kaplan-Meier analysis revealed that patients with 3 elevated biomarkers had a significantly higher cardiac event rate than patients with a lower number of elevated biomarkers.

**Conclusion**

The combination of these 3 biomarkers could reliably risk-stratify CHF patients for prediction of cardiac events. (Circ J 2008; 72: 1800–1805)

**Key Words:** Biomarkers; Chronic heart failure; Risk stratification

**C**hronic heart failure (CHF) is still a major cause of death and hospitalization, and has a poor prognosis despite the significant reduction in mortality achieved in clinical trials. Therefore, the prognostic evaluation and risk stratification of CHF patients continues to increase in importance and involves a complex assessment of multiple interacting variables.

Several new cardiac biomarkers have emerged as strong predictors of risk among CHF patients. Importantly, these biomarkers are mainly divided into 3 different pathophysiological aspects: (1) neurohormonal markers that reveal pressure and/or volume overload, (2) markers of myocardial damage, and (3) markers of inflammation. B-type natriuretic peptide (BNP) is secreted from the ventricles by mechanical overload and is the most established marker of neurohormonal factors. Heart-type fatty acid-binding protein (H-FABP) is a novel marker of ongoing myocardial cell injury and pentraxin 3 (PTX3) is a novel marker of inflammation. These 3 biomarkers have been used for predicting cardiac events in CHF patients but the incremental usefulness of the combination of these 3 biomarkers has not been previously examined in patients with CHF. Recently, a multi-axis framework has been proposed in order to more completely appreciate the mechanisms of cardiovascular diseases. Thus, we hypothesized that the combination of 3 biomarkers would provide complementary information and stratify risk more effectively among patients with CHF.

**Methods**

**Study Design**

We prospectively studied 164 consecutive patients (92 men, 72 women; mean age, 68±14 years) who were admitted to the Yamagata University Hospital from April 1996 to February 2005 for the treatment of worsening CHF, for diagnosis and pathological investigation of heart failure, or for therapeutic evaluation of heart failure. The diagnosis of CHF was based on 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 radionuclide ventriculography. Baseline characteristics of the study subjects are listed in Table 1. The diagnoses of hypertension, diabetes, and hyperlipidemia were obtained from medical records or patient history of currently or previously received medical therapy. Exclusion criteria in this study were those with clinical or electrocardiographic evidence suggestive of acute coronary syndrome within the 3 months preceding admission, those with renal insufficiency characterized by a serum creatinine concentration of 2.5 mg/dl or greater, and those with a history of malignancy or severe liver disease.

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(Received February 14, 2008; revised manuscript received June 2, 2008; accepted July 2, 2008; released online October 3, 2008)
>1.5 mg/dl, those with inflammatory disease and any documented inflammatory illness such as arthritis, connective tissue disease, active hepatic disease, pulmonary disease, or any malignancy. Informed consent was given by all patients before participation in this study, and the protocol was approved by the institution’s Human Investigations Committee.

Blood samples were obtained on admission for measurement of plasma BNP, serum H-FABP, and plasma PTX3 levels. The optimal cut-off values for the 3 biomarkers were determined as those with the largest sum of sensitivity plus specificity on each of the receiver-operating characteristic (ROC) curves. Cut-off values of BNP (200 pg/ml), H-FABP (4.1 ng/ml), and PTX3 (4.0 ng/ml) were determined by ROC curves as shown in Fig. 1. Patients were categorized into 4 groups on the basis of the number of elevated biomarkers (score 0–3). Glomerular filtration rate (GFR) was estimated from the modification of diet in renal disease equation modified by a Japanese coefficient.6

Transthoracic echocardiography was performed by experienced echocardiologists without knowledge of the biochemical data, using an ultrasound instrument (Hewlett Packard SONOS 7500, Palo Alto, CA, USA) equipped with a sector transducer (carrier frequency of 2.5 or 3.75 MHz) within 1 week after admission.

Endpoints and Follow-up
No patients were lost to follow-up (mean follow-up 679±438 days) after admission. Events were centrally adjudicated using medical records, autopsy reports and death certificates. The endpoints, which were judged independently by researchers, were (1) cardiac death, defined as death from worsening heart failure or sudden cardiac death, and (2) worsening heart failure requiring readmission. Sudden cardiac death was defined as death without definite premonitory symptoms or signs and was established by the attending physician.

Assays of BNP, H-FABP, and PTX3

**BNP** The venous blood samples were transferred to chilled tubes containing 4.5 mg of ethylenediaminetetraacetic acid disodium salt and aprotinin (500 U/ml), and immediately centrifuged at 1,000 G for 15 min at 4°C to examine plasma biomarkers. The clarified plasma samples were frozen, stored at −70°C and thawed just before assay. Plasma BNP levels were measured using a commercially available specific radioimmunoassay for human BNP (Shiono RIA BNP assay kit, Shionogi Co Ltd, Tokyo, Japan)6

**H-FABP** The venous blood samples were immediately centrifuged at 2,500 G for 15 min at 4°C to measure serum H-FABP levels. The clarified serum samples were frozen, stored at −70°C, and thawed just before assay. H-FABP levels were measured using a 2-step sandwich enzyme-linked immunosorbent assay (ELISA) kit (MARKIT-M H-FABP, Dainippon Pharmaceutical Co Ltd, Tokyo, Japan) as previously reported.6

**PTX3** Plasma PTX3 levels were measured using the ELISA kit (Perseus Proteomics Inc, Tokyo, Japan) as previously reported.7

The analytical range of these kits was 4.0–2,000 pg/ml for BNP assay, 1.1–250 ng/ml for H-FABP assay, and 0.1–20 ng/ml for PTX3 assay.

**Statistical Analysis**
Results are presented as mean±standard deviation (SD) values for continuous variables and as the percentage of total patients for categorical variables. Skewed variables are presented as median and interquartile range. Unpaired Student’s t-test and the chi-square test were used for comparisons between 2 groups of continuous and categorical variables, respectively. If data were not distributed normally, the Mann-Whitney U-test was used. Comparison of data
among 4 groups categorized on the basis of the number of elevated biomarkers was performed by the Kruskal-Wallis test. A Cox proportional hazard analysis was performed to evaluate the associations between cardiac events and measurements. The cardiac event-free curve was computed according to the Kaplan-Meier method and compared by the log-rank test. The ROC curves were constructed to illustrate various cut-off values of BNP, H-FABP and PTX3 for predicting cardiac events at 36 months and to determine optimal sensitivity and specificity. All p-values reported are 2-sided, and p<0.05 was considered significant. Statistical analysis was performed with a standard statistical program package (StatView, version 5.0, SAS Institute Inc, Cary, NC, USA).

### Result

**Patient Characteristics**

The baseline characteristics of 164 heart failure patients are shown in Table 1. The mean age of study subjects was 68±14 years old, 56% of patients were men and 36% were in New York Heart Association (NYHA) functional class III or IV. The etiologies of heart failure were dilated cardiomyopathy in 27% and ischemic heart disease in 23% (Table 1). A simple scoring system based upon the number of elevated biomarkers was 1.6±1.1. Correlations of these markers were very weak (BNP and H-FABP: R=0.342, p<0.0001; BNP and PTX3: R=0.235, p=0.0025; H-FABP and PTX3: R=0.216, p=0.0054), suggesting that these markers reflect different features of the pathophysiologic process of heart failure.

### Clinical Outcomes

All patients were followed-up completely. There were 49 cardiac events (30%), comprising 18 cardiac deaths and 31 re-hospitalizations for worsening heart failure during the follow-up period. As shown in Table 2, patients with cardiac events were older, and had more severe NYHA functional class than those without cardiac events. Furthermore, patients with...
cardiac events showed higher levels of uric acid, BNP, H-FABP, PTX3, lower estimated GFR (eGFR), and higher score based upon the number of elevated biomarkers compared with those without cardiac events, whereas other parameters, including gender and numbers of patients who had hypertension, diabetes mellitus, hyperlipidemia, or were currently smoking, were not significantly different between patients with and without cardiac events.

The univariate Cox proportional hazard analysis to predict cardiac events is shown in Table 3. Age, NYHA classification, eGFR, uric acid, BNP, H-FABP, and PTX3 were related significantly to cardiac events. Those variables with p-values less than 0.05 were entered into the multivariate Cox proportional hazard regression analysis (Table 4), and only PTX3 was an independent predictor of future cardiac events.

**Classification by Number of Elevated Biomarkers**

Patients were categorized into 4 groups (score 0–3) on the basis of the number of elevated biomarkers (Table 5). Cut-off values of BNP (200 pg/ml), H-FABP (4.1 ng/ml), and PTX3 (4.0 ng/ml) were determined by ROC curves, as shown in Fig 1. The number of patients over each cut-off value determined by the ROC curves was 95 (58%) for BNP >200 pg/ml, 84 (51%) for H-FABP >4.1 ng/ml, and 81 (49%) for PTX3 >4.0 ng/ml.

Patients with score 3 were older, and had a more severe NYHA functional class, lower left ventricular ejection fraction, lower eGFR, and higher levels of uric acid, BNP, H-FABP, and PTX3 compared with those with score 0–2 (Table 5). Furthermore, patients with score 3 had significantly higher rates of rehospitalization and cardiac death than those with score 0–2 (p<0.0001; Fig 2A). Other parameters, including gender and etiology of CHF, were not significantly different among the 4 groups. In addition, there was no difference among the 4 groups in the numbers of patients who had hypertension, diabetes mellitus, hyperlipidemia, or were currently smoking.

Kaplan-Meier analysis demonstrated that patients with score 3 had significantly higher cardiac event rates than patients with score 0–2 (Fig 2B). These results suggest that when the number of elevated biomarkers is high, prognosis is poor and intense follow-up after discharge with chest X-ray, echocardiography, and blood examination is recommended.

**Risk Stratification by Number of Elevated Biomarkers**

Prognostic results of the univariate Cox proportional hazard analysis to predict cardiac events are shown in Fig 3: patients with score 1, 2, and 3 had a 5.4-fold, 11.2-fold

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**Table 4** Results of Multivariate Cox Proportional Hazard Analysis

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio</th>
<th>95%CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, 5-year increase</td>
<td>1.08</td>
<td>0.96–1.23</td>
<td>NS</td>
</tr>
<tr>
<td>NYHA I/II vs III/IV</td>
<td>1.69</td>
<td>0.79–3.59</td>
<td>NS</td>
</tr>
<tr>
<td>eGFR, 20.1-ml increase</td>
<td>0.96</td>
<td>0.67–1.40</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid, 2.0-mg/dl increase</td>
<td>1.14</td>
<td>0.86–1.50</td>
<td>NS</td>
</tr>
<tr>
<td>BNP, 758-pg/ml increase</td>
<td>1.05</td>
<td>1.00–1.08</td>
<td>NS</td>
</tr>
<tr>
<td>H-FABP, 5.5-ng/ml increase</td>
<td>1.29</td>
<td>0.91–1.83</td>
<td>NS</td>
</tr>
<tr>
<td>PTX3, 4.7-ng/ml increase</td>
<td>1.24</td>
<td>1.01–1.53</td>
<td>0.0458</td>
</tr>
</tbody>
</table>

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**Table 5** Clinical Characteristics of the 4 Groups of CHF Patients

<table>
<thead>
<tr>
<th>No. of elevated biomarkers</th>
<th>Score 0 (n=33)</th>
<th>Score 1 (n=46)</th>
<th>Score 2 (n=41)</th>
<th>Score 3 (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60±10</td>
<td>69±11**</td>
<td>66±18*</td>
<td>75±10**,#††</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>21/12</td>
<td>24/22</td>
<td>23/18</td>
<td>24/20</td>
</tr>
<tr>
<td>Hypertension</td>
<td>17 (52%)</td>
<td>22 (48%)</td>
<td>23 (56%)</td>
<td>19 (43%)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>7 (21%)</td>
<td>12 (26%)</td>
<td>5 (12%)</td>
<td>11 (25%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7 (21%)</td>
<td>14 (30%)</td>
<td>12 (29%)</td>
<td>7 (16%)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>7 (21%)</td>
<td>8 (17%)</td>
<td>8 (20%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>I/II 33 (100%)</td>
<td>36 (78%)</td>
<td>21 (51%)</td>
<td>14 (32%)†</td>
</tr>
<tr>
<td>II/IV</td>
<td>0 (0%)</td>
<td>10 (22%)</td>
<td>20 (49%)</td>
<td>30 (68%)†</td>
</tr>
<tr>
<td>Etiology of heart failure</td>
<td>IHD 6 (18%)</td>
<td>11 (24%)</td>
<td>10 (24%)</td>
<td>11 (25%)</td>
</tr>
<tr>
<td>Non-IHD</td>
<td>27 (82%)</td>
<td>35 (76%)</td>
<td>34 (79%)</td>
<td>33 (75%)</td>
</tr>
<tr>
<td>Echocardiography</td>
<td>LVESD (mm)</td>
<td>52.8±8.5</td>
<td>53.5±9.2</td>
<td>54.3±7.9</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>63.0±12.8</td>
<td>50.0±15.0**</td>
<td>48.5±17.6**</td>
<td>40.6±2.5***</td>
</tr>
<tr>
<td>Laboratory data</td>
<td>eGFR (ml·min⁻¹·1.73m⁻²)</td>
<td>76.1±18.8</td>
<td>67.1±18.5*</td>
<td>65.5±20.0*</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>5.9±2.1</td>
<td>6.1±2.0</td>
<td>5.8±1.8</td>
<td>6.9±2.0*</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>68 (35–112)</td>
<td>138 (56–294)</td>
<td>410 (233–764)<strong>,## 1.035 (555–1685)</strong>,##,††</td>
<td></td>
</tr>
<tr>
<td>H-FABP (ng/ml)</td>
<td>2.8 (2.1–3.4)</td>
<td>4.1 (3.1–4.8)</td>
<td>4.5 (2.9–6.8)*</td>
<td>7.6 (6.1–11.0)**,##,††</td>
</tr>
<tr>
<td>PTX3 (ng/ml)</td>
<td>2.0 (1.7–2.5)</td>
<td>2.8 (2.0–3.7)</td>
<td>5.0 (3.2–7.0)**,##</td>
<td>6.8 (5.1–10.9)**,##,††</td>
</tr>
</tbody>
</table>

Skewed data are reported as median (interquartile range).

*p<0.05, **p<0.01 vs score 0; #p<0.05, ##p<0.01 vs score 1; †p<0.05, †† p<0.01 vs score 2; p<0.01 by chi-square test.

Abbreviations as in Tables 1,3.
(p<0.05), and 34.6-fold increase (p<0.01), respectively, in the risk of adverse cardiac events compared with those with score 0 (Fig 3A). When patients were categorized by BNP, H-FABP, and NYHA classification, patients with score 1, 2, and 3 had a 4.8-fold, 9.1-fold, and 16.9-fold increase, respectively, in the risk of adverse cardiac events compared with score 0 (Fig 3B). In addition, an increase of one score had a hazard ratio of 2.801 in the combination of BNP, H-FABP, and PTX3, and hazard ratio of 2.142 in the combination of BNP, H-FABP, and NYHA classification. These data clearly demonstrate that the combination of BNP, H-FABP, and PTX3 can stratify risk of CHF more effectively than the combination of BNP, H-FABP and NYHA class.

**Discussion**

We have shown that the number of elevated biomarkers (BNP, H-FABP, and PTX3) was significantly higher in patients with cardiac events than in those without cardiac events. Because CHF is a major public health problem, it is necessary to grade the severity of CHF patients. This study examined whether the combination of BNP, H-FABP, and PTX3 provides valuable information for risk stratification in patients with CHF. Univariate Cox proportional hazard analysis demonstrated that patients with 3 elevated biomarkers were associated with the highest risk (34.6-fold) for cardiac events compared with patients with a lesser number of elevated biomarkers. Furthermore, Kaplan-Meier analysis demonstrated that cardiac events occurred most frequently in patients with 3 elevated biomarkers compared with patients with less than 3 elevated biomarkers. These results suggest that the combination of these 3 biomarkers could improve risk stratification for the prediction of cardiac events in CHF patients.

Because CHF is accompanied by a variety of pathological changes that trigger disease progression, a multi-axis framework has been proposed in order to more effectively appreciate the pathophysiology of CHF. Therefore, the combination of multiple biomarkers, which is a novel method of risk stratification of CHF patients, may provide helpful information for understanding different aspects of the interrelated pathophysiological processes of CHF.

We selected 3 different pathogenic features (neurohormonal markers, markers of myocardial damage, and markers of inflammation) on the basis of previous clinical studies. BNP is secreted from the ventricles by mechanical overload and is a well-established prognostic factor in CHF patients. H-FABP is abundant in the cytosol of cardiomyocytes and is released into the circulation when the cell surface membrane is injured. H-FABP levels are increased in patients with advanced CHF because of leakage of cytosolic proteins from cardiomyocytes affected by the ongoing myocardial damage. We previously reported that H-FABP was more sensitive than troponin T, a myofibrillar component, for detecting ongoing myocardial damage.

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Fig 2. Cardiac mortality and all cardiac events. (A) Patients with score 3 had the highest rates of rehospitalization and cardiac death among the 4 scoring groups. **p<0.001 by chi-square test. (B) Kaplan-Meier analysis in chronic heart failure patients stratified into 4 groups based on the number of elevated biomarkers. Patients with score 3 had significantly higher rates of cardiac events than patients with score 0–2.

Fig 3. Hazard ratios to predict cardiac events. Univariate Cox proportional hazard analysis demonstrated that patients with score 3 were associated with the highest risk for cardiac events among 4 groups. Patients were categorized by B-type natriuretic peptide (BNP), heart-type fatty acid-binding protein (H-FABP), and pentraxin 3 (A) and by BNP, H-FABP, and New York Heart Association classification (B). *p<0.05 and **p<0.01 vs patients with score 0.
Recently it was reported that inflammatory markers, such as high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor-alpha (TNF-α), were related to decreasing functional status and provide important prognostic information about the morbidity and mortality of CHF patients. PTX3 is in the pentraxin superfamily and a newly discovered marker of the acute-phase inflammatory response. We previously showed that PTX3 was superior to hs-CRP and TNF-α in predicting cardiac events of CHF patients; so in the present study we examined the possibility of combining the measurement of BNP, H-FABP, and PTX3 to reflect different aspects of CHF. Improved risk stratification of CHF patients may depend on the discovery of new biomarkers. In addition, it is necessary in a future study to find treatments to reduce these biomarkers and improve clinical outcomes.

Conclusions
Our data suggest that measuring the combination of BNP, H-FABP, and PTX3 is highly reliable method for risk stratification of patients hospitalized for CHF. It is necessary to examine in a future study whether this approach allows clinicians to improve the management of CHF patients.

Acknowledgments
This study was supported in part by a grant-in-aid for Scientific Research (No. 19590804 and 19790513) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References