Relationship Between Biological Variation in B-Type Natriuretic Peptide and Plasma Renin Concentration in Stable Outpatients With Dilated Cardiomyopathy

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Background: The aim of the present study was to interpret B-type natriuretic peptide (BNP) level in outpatients with stable chronic heart failure (CHF); it is important to clarify whether the change in BNP represents disease progression or a range of biological variation.

Methods and Results: To compare biological variation in BNP and biological variation in factors of the renin-angiotensin-aldosterone system (RAS) in stable CHF patients with dilated cardiomyopathy (DCM), the BNP plasma levels and RAS factors were measured in 115 stable outpatients with DCM. According to stepwise multivariate analysis, plasma BNP at baseline (P=0.005), presence of atrial fibrillation (P=0.015), and a high biological variation in plasma renin concentration (PRC; P=0.002) were significant independent dominant factors related to a high biological variation in BNP. Although there was no change in body weight or blood pressure during the 2-month study period, the % change in hematocrit was negatively correlated with % change in BNP (r=-0.327, P=0.0008), and positively correlated with % change in PRC (r=0.671, P=0.001).

Conclusions: There was a significant relationship between biological variation in BNP and biological variation in PRC, suggesting that the physiological interaction between the natriuretic peptide system and RAS may contribute to the biological variation in plasma BNP in stable outpatients with DCM. (Circ J 2011; 75: 1897–1904)

Key Words: Biological variation; Brain natriuretic peptide; Dilated cardiomyopathy; Heart failure; Renin concentration

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Table 1. Baseline Patient Characteristics (n=115)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Characteristics</th>
<th>LVEF (%)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>BMI (kg/m²)</td>
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</tr>
<tr>
<td>60.7±9.7</td>
<td>24.9±2.8</td>
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<tr>
<td>Gender (M/F)</td>
<td>107/8</td>
<td></td>
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<tr>
<td>NYHA functional class (I/II/III)</td>
<td>20/67/28</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>110.1±14.3</td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>44.7±14.3</td>
<td></td>
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<tr>
<td>AF, n (%)</td>
<td>33 (28.6)</td>
<td></td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>66.8±10.8</td>
<td></td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>66.2±8.9</td>
<td></td>
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<tr>
<td>LVDd (mm)</td>
<td>64.8±8.6</td>
<td></td>
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<tr>
<td>Baseline therapy</td>
<td></td>
<td></td>
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<tr>
<td>ACE-I or ARB</td>
<td>13 (98.2)</td>
<td></td>
</tr>
<tr>
<td>β-blockers, n (%)</td>
<td>108 (93.9)</td>
<td></td>
</tr>
<tr>
<td>Spironolactone, n (%)</td>
<td>101 (87.8)</td>
<td></td>
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<tr>
<td>Loop diuretics, n (%)</td>
<td>82 (71.3)</td>
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</table>

BMI, body mass index; NYHA, New York Heart Association; AF, atrial fibrillation; HR, heart rate; BP, blood pressure; LVDd, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

There was no significant relationship between biological variation in BNP and biological variation in vasoconstrictor neurohumoral factors in patients with CHF. Therefore, we evaluated the relationship between biological variability in BNP and biological variability in biomarkers in RAS in stable outpatients with dilated cardiomyopathy (DCM).

**Methods**

**Patients**

A total of 121 consecutive outpatients with DCM who remained clinically stable for more than 6 months after discharge were enrolled in the present study. To be eligible for the study, patients with DCM were required to have been previously admitted to hospital for management of CHF due to systolic heart failure, which was defined as left ventricular ejection fraction (LVEF) <45% (at admission) on 2-D echocardiography or ventriculography using contrast medium or radioisotope. The diagnosis of DCM was based on patient history, physical examination, electrocardiography, chest radiology, echocardiography, left ventriculography coronary angiography and endomyocardial biopsy. Endomyocardial biopsies were obtained to rule out secondary cardiomyopathies caused by viral or other infectious myocarditis, sarcoidosis, amyloidosis or other metabolic heart disease. We excluded patients with renal failure (serum creatinine >2.0 mg/dl), chronic inflammatory disease or malignancy.

Patients were treated according to heart failure guidelines23 by a heart failure specialist after discharge. Informed consent was obtained from all patients for participation in the study according to a protocol approved by the Committee on Human Investigation at Shiga University Hospital.

**Study Protocol**

This was a prospective study in stable outpatients with DCM. Strict stable clinical conditions during the 2-month study period were defined as: stable body weight (<±1 kg), unchanged New York Heart Association (NYHA) functional class, unchanged heart failure medication, and unchanged LVEF (<±5%). At entry, blood samples were drawn from the antecubital vein, after at least 30 min of bed rest in a supine position, and LVEF was measured on echocardiography at the same time, and each patient was requested to visit the hospital between 13:00 and 16:00 h. After all patients had been followed for 2 months, plasma levels of neurohumoral factors and LVEF were re-evaluated. LVEF was measured again by the same sonographer. On echocardiography, all patients had LVEF measured using the biplane disc summation method (Simpson’s rule; mean LVEF at baseline=44.7±8.6%). A heart failure specialist blinded to the plasma levels of neurohumoral factors until study completion managed patients according to standard regimens, based on improvement of symptoms, physical examination and pulmonary congestion on chest X-ray.

In addition, because biological variation could not be either an increase or decrease, we also evaluated % change (at 2 months) because the % changes may provide important information for understanding the mechanism of wide biological variations in BNP.

**Measurement of Neurohumoral Factors**

The blood samples were immediately placed on ice and centrifuged at 4°C, and the plasma was frozen in aliquots and stored at −30°C until assay. In the present study all blood samples were evaluated at the end of the observation term under the same lot number in order to avoid an error margin among lots. Plasma BNP concentrations were measured using an automated chemiluminescent enzyme immunoassay analyzer exclusive kit (Shionogi, Osaka, Japan) as previously reported10.24.28 and the intra- and inter-assay coefficients of variation were 1.3% (n=6) and 1.9% (n=6), respectively. The PRC was measured using a specific and sensitive immunoradiometric assay kit (SRL, Tokyo, Japan) as previously reported16 and the intra- and inter-assay coefficients of variation were 1.4% (n=10) at 74 pg/ml and 1.5% (n=10) at 72 pg/ml, respectively. The plasma levels of Ang II, ALD and norepinephrine were measured as previously reported27.30

**Statistical Analysis**

All results are expressed as mean±SD or median (interquartile range, IQR). In the present study we defined the biological variation as total coefficient of variation (CVt). The CVt was calculated as the baseline-to-2-month coefficient of variation (=SD/mean). The CVt and the analytical coefficient of variation (CVa) provided the basis for individual biological coefficient of variation (CVi), given as CVi=CVt−CVa/2 as previously reported.16,22 Then % changes were calculated as (level at 2 months−level at baseline)/level at baseline×100%. Changes in continuous variables between baseline and at 2 months were tested for significance using Student’s t-test (parametric data) and Wilcoxon signed-rank test (non-parametric data) for paired data. Associations between biological variations in BNP and study variables were examined using Pearson’s correlation coefficient and linear regression. Multivariate linear regression analysis was performed to examine independent correlates of biological variations in BNP, including baseline variables that were associated with biological variations in BNP at P<0.10 on univariate analysis. The β-coefficients were standardized regression coefficients. P<0.05 was considered significant.
Results

Baseline Patient Characteristics
Of stable outpatients with CHF, 2 patients were rehospitalized, 2 patients required a change in heart failure medication, and 2 patients had a change in LVEF (>±5%) during the first 2 months. Finally, the remaining 115 outpatients who remained clinically stable for 2 months were enrolled in the present study. There were 87 patients in NYHA functional class I or II, and 28 patients in class III (Table 1). Most patients were already receiving the standard oral therapy.

Changes in Clinical and Laboratory Data vs. Time
In the present study, none of the 115 outpatients had a significant change in heart rate, mean blood pressure, body weight, hematocrit, serum creatinine, BNP, norepinephrine, or RAS factors during the 2-month study period (Tables 2, 3). The median absolute change in BNP (level at 2 months – level at baseline) was 7.7% (IQR, –25.8 to 35.9%; CVt = 21.1% vs. 21.1 ± 14.5, P=0.002), and patients with sinus rhythm (28.0±21.7% vs. 20.5±16.9%, P=0.004). Analytical and biological variations of biochemical variables are given in Table 3.

Correlations of Individual Biomarkers With Biological Variation
Biological variation in BNP was positively related to biological variation in PRC (r=0.334, P=0.002), Ang II (r=0.318, P=0.006), and ALD (r=0.235, P=0.007). In addition, there was a significant correlation between biological variation in BNP and biological variation in hematocrit (r=0.280, P=0.002; Figure 2).

Univariate and Multivariate Linear Regression Model of Biological Variations in BNP in Stable Outpatients With DCM
On univariate analysis, 6 clinical, neurohumoral factors were significant predictors of high biological variation in BNP (Table 4). On stepwise multivariate analysis, low plasma BNP concentration at baseline (P=0.005), presence of AF (P=0.015), and high biological variation in PRC (P=0.002) were significant independent predictors of high biological variation in BNP.

Relationships Among % Change in BNP, PRC, Ang II, ALD, and Hematocrit in Stable Outpatients With DCM
The % change in hematocrit was negatively correlated with % change in BNP (r=–0.327, P=0.0008), and positively correlated with % change in PRC (r=0.671, P<0.001; Figure 3). The % change in BNP was negatively related to % change of
Figure 1. (A) Correlation between plasma B-type natriuretic peptide (BNP) at baseline and biological variations in BNP. (B) Comparison of absolute changes in BNP (at 2 months) and % change in BNP (at 2 months) with regard to baseline BNP in stable outpatients with chronic heart failure.

Figure 2. Correlation between biological variation in B-type natriuretic peptide (BNP; at 2 months) with biological variation in hematocrit (Ht; at 2 months), and with biological variation in plasma renin concentration (PRC; at 2 months) in stable outpatients with chronic heart failure.
Biological Variations of BNP and Renin in CHF

PRC \( r=-0.256, P=0.009 \), Ang II \( r=-0.303, P=0.002 \), and ALD \( r=-0.421, P=0.001 \); Figure 4), suggesting that changes of circulating volume may influence changes of BNP and RAS in stable outpatients with CHF due to DCM.

**Discussion**

Serial BNP data are not well established, due to poor evidence partly caused by biological variation in plasma BNP. In the present study, we evaluated the clinical factors that may influence biological variations in plasma BNP in stable outpatients with DCM. In the present study, we have shown for the first time that there is a significant relationship between biological variation in BNP and biological variation in RAS factors in patients with CHF.

The \% change in BNP was negatively related to \% change in hematocrit, and the \% change in hematocrit was positively correlated with \% change of PRC (Figure 3). In addition, the \% change in BNP was negatively related to \% change in RAS factors (Figure 4), and a high biological variation in PRC was a significant independent predictor of high biological variation in BNP, suggesting that the changes of circulating volume or the changes of distribution of circulating volume without changes of body weight and blood pressure (Table 2) may contribute to the changes of BNP and RAS in stable outpatients with DCM.

In clinical practice, physicians may consider that circulating volume is stable in stable outpatients if there is no change of body weight and blood pressure. In this situation, the unexpected changes of BNP when assessing CHF may be considered variations. Although we did not measure circulation volume or filling pressure, the negative correlation between \% change in hematocrit and \% change in BNP after 2 months strongly suggests that changes of BNP are sensitive to circulating volume in individual patients. If the change of circulating volume is determined from hematocrit and BNP, the reason for the lack of significant changes in blood pressure after 2 months may be due to the compensatory activation of the RAS factors.

Bruins et al showed that intra-day, day-to-day and week-

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**Table 4. Dominant Factors of Biological Variations in BNP in Stable Outpatients With DCM**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Univariate correlation coefficients</th>
<th>P value</th>
<th>Multivariate ( \beta )-coefficients</th>
<th>P value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>0.017</td>
<td>0.852</td>
<td></td>
<td></td>
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<tr>
<td>Gender (Male=1)</td>
<td>-0.124</td>
<td>0.188</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>-0.138</td>
<td>0.141</td>
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<tr>
<td>HR (beats/min)</td>
<td>0.101</td>
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<td>MBP (mmHg)</td>
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<td>AF (yes=1 no=0)</td>
<td>0.342</td>
<td>0.002</td>
<td>0.217</td>
<td>0.015</td>
</tr>
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<td>LVEF (%)</td>
<td>-0.142</td>
<td>0.138</td>
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<tr>
<td>BNP (pg/ml)</td>
<td>-0.230</td>
<td>0.0136</td>
<td>-0.243</td>
<td>0.005</td>
</tr>
<tr>
<td>Biological variations</td>
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<td></td>
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<tr>
<td>Creatinine (%)</td>
<td>0.154</td>
<td>0.148</td>
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<tr>
<td>Hematocrit (%)</td>
<td>0.280</td>
<td>0.002</td>
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<tr>
<td>PRC (%)</td>
<td>0.334</td>
<td>0.002</td>
<td>0.276</td>
<td>0.002</td>
</tr>
<tr>
<td>Ang-II (%)</td>
<td>0.318</td>
<td>0.006</td>
<td></td>
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<tr>
<td>ALD (%)</td>
<td>0.235</td>
<td>0.012</td>
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</tbody>
</table>

Abbreviations as given in Tables 1, 2.

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**Figure 3.** Percentage change in B-type natriuretic peptide (BNP; at 2 months) and \% change in plasma renin concentration (PRC; at 2 months) with regard to \% change in hematocrit (Ht; at 2 months) in stable outpatients with chronic heart failure.
to-week biological variations in BNP increase with the interval between peptide measurements (12%, 27% and 41%, respectively), and biologic variations may therefore be time dependent. In addition, Schou et al showed that the week-to-week biological variation in BNP was 16%, under stable conditions in CHF patients selected according to strict criteria. In the present study, biological variation in BNP after 2 months was calculated as 21.3%. There are no previous studies that have prospectively evaluated biological variations in vasoconstrictor biomarkers such as plasma norepinephrine, PRC, Ang II, or ALD in pre-specified stable outpatients with CHF. In the present study, biological variations in norepinephrine, PRC, Ang II and ALD have been calculated for the first time as 16.6%, 39.9%, 29.3% and 18.4%, respectively. We estimated that plasma levels of norepinephrine and biomarkers of RAS are strongly influenced by the sampling condition and medications, therefore blood samples were drawn from the antecubital vein, after at least 30 min of bed rest in a supine position between 13.00 and 16.00 h, and treatments were not changed before and after 2 months. As shown in the present study, biological variations in these vasoconstrictors were not so high compared with that of BNP (21.3%).

Biological variations in BNP were significantly higher in CHF patients with AF than in CHF patients without AF. There was no difference in baseline characteristics, including CHF severity and BNP levels, between CHF patients with and without AF (180.2±219 pg/ml vs. 144.3±173 pg/ml, P=0.09). After adjustment of clinical variables, the presence of AF was a significant independent predictor of high biological variation in BNP. The reason why biological variation in BNP was significantly higher in patients with AF than in those with sinus rhythm remains unclear; an irregular R-R interval may contribute to the biological variations in BNP.

Study Limitations

There were several limitations in the present study. In the clinical setting it is often difficult to determine whether increase in BNP level is within normal biological variation or not. Indeed, the variations in BNP described herein may involve both within-person biological variation and disease progression. In the present study, there was a significant relationship between biological variation in BNP and biological variation in RAS factors in patients with CHF. In addition, in a recent study we showed that both an absolute high BNP level (>189 pg/ml) and % increase in BNP (>15%, 2-month interval) provided important prognostic information in clinically stable outpatients with non-ischemic heart failure, suggesting that not only BNP level but also % changes in BNP are important for evaluating severity of CHF. Therefore, changes in BNP may be a sensitive biomarker of CHF. Future large-scale study is necessary, however, to evaluate the biological variation in plasma BNP in outpatients with stable CHF and prognosis.
The 2-month time interval for sequential measurements of natriuretic peptides was selected based on consideration of past reports, and the average interval between hospital visits for outpatients with DCM at Shiga University Hospital. Furthermore, biological variations in the degrees of exertion or volume/food intake prior to the day of measurement are difficult to quantify and may account for some of the variability. Attempts were made to avoid this, however, by taking all samples at the same time of day (in the afternoon), and blood samples were drawn from the anteceubital vein after at least 30 min of bed rest in a supine position. Bruins et al showed that plasma BNP concentrations increase during the day and stabilize in the afternoon. We could not measure circulating volume or filling pressure and we estimated change of circulating volume using change of hematocrit, which may not be a reliable marker of the change of circulating volume. We also measured the % change of blood urea nitrogen/creatinine ratio, which was correlated with % change of BNP (data not shown). Further studies are needed, however, to evaluate changes of circulating volume or filling pressure to clarify the present hypothesis.

Conclusion

There was a significant relationship between biological variation in BNP and biological variation in the RAS factors, suggesting that the physiological interaction between the natriuretic peptide system and RAS reflected by the changes of circulating volume or the changes of distribution of circulating volume may contribute to the biological variation in plasma BNP in stable outpatients with DCM.

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References


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