Plasma B-type Natriuretic Peptide Levels and Risk Factors for Congestive Heart Failure in a Japanese General Population

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SUMMARY

This cross-sectional study was performed to establish the rationale for BNP testing for identifying subjects at high risk of congestive heart failure (CHF) in a screening setting. Plasma BNP concentrations were measured in 8,178 community-dwelling residents (mean age, 62 ± 12 years; 3,194 males). First, in order to determine age- and sex-related reference values for plasma BNP levels, subjects having factors known to influence plasma BNP levels were excluded. The remaining 3,410 subjects were eligible for the reference study. Second, to verify BNP testing for screening for subjects at high risk of CHF, the clinical characteristics of subjects showing abnormally high plasma BNP levels (≥ 97.5 percentile for each age- and sex-specific value of the reference cohort) were examined.

In the reference subjects, plasma BNP levels increased with age in both genders, and were higher in women than in men. In the original cohort, age- and sex-specific reference values for high plasma BNP levels were related to the presence of major ECG abnormalities, hypertension, mildly elevated serum creatinine levels, and a history of coronary heart disease.

The results of the present study indicate that individuals with high plasma BNP levels in the community have accumulating risk factors for CHF. This suggests that plasma BNP measurement may be a useful screening test for identification of individuals at high risk of CHF within a Japanese general population. (Int Heart J 2005; 46: 465-475)

Key words: Brain natriuretic peptide, Congestive heart failure, Screening test

The prevalence of congestive heart failure (CHF) is growing with an increase in the mean age of the population. Once overtly manifest, CHF is an extremely lethal condition associated with a very poor quality of life and prognosis. The

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mortality rate after the onset of CHF remains high despite recent advances in the management of this condition. 1) Because most patients with CHF have several modifiable risk factors, 2) it is important to target its preclinical stages and treat known risk factors before the development of overt CHF. It is therefore essential that a screening test be established to identify individuals at high risk of CHF early enough to prevent or postpone progression to overt CHF. 3)

B-type natriuretic peptide (BNP) is a cardiac neurohormone secreted from the myocardium in response to changes in intracardiac volume and pressure. 4, 5) Plasma BNP levels are known to be elevated in patients with symptomatic left ventricular systolic dysfunction 6, 7) and correlate to New York Heart Association (NYHA) class as well as prognosis. 8, 9) In addition, irrespective of the degree of left ventricular dysfunction, plasma BNP levels have been shown to be elevated in patients with various cardiac disorders including previous myocardial infarction, cardiomyopathy, valvular heart disease, hypertensive heart disease, and atrial fibrillation. 6, 10-13) It is therefore possible that measurement of plasma BNP levels might be a potential screening marker for identifying individuals with asymptomatic CHF as well as those at high risk of CHF due to various forms of structural heart disease. 14, 15)

These suggest that plasma BNP levels might be elevated in subjects with precursors of CHF and thus may serve as a useful predictor for new onset of CHF in a screening setting in the general population. However, in the general population, recent reports have shown that the plasma BNP level is affected by extracardiac factors such as estrogen, 16) obesity, 17) and genetics. 18) In fact, median plasma BNP levels in healthy subjects are clearly higher in women than men. 16, 19) This may contradict epidemiological evidence indicating a higher prevalence of cardiovascular disorders in men. These observations suggest that plasma BNP measurement might not be an optimal marker with which to identify subjects at high-risk of CHF in mass-screening. Although only one report, the Framingham study, has shown that a subject group with plasma BNP levels in the highest third of the range measured exhibited an incidence of CHF several times higher than that in the lowest group, 20) no studies have demonstrated whether plasma BNP is a sensitive marker for subjects at high-risk of CHF and thus a predictor of new onset CHF in the Japanese general population.

The objective of this cross-sectional study was to validate the hypothesis that a high plasma BNP level is a useful marker of individuals at high risk of CHF within the Japanese general population.
METHODS

Study population: The Iwate-Kenpoku Cohort (Iwate KENCO) study was designed to prospectively investigate the risks of CHF, acute myocardial infarction, and stroke in a general adult population in northern Japan. The sample for the present investigation consisted of 8,178 participants (male; 2,906; female; 5,272) who participated in this cohort study between April and December 2002. All participants gave written informed consent. The study protocol was approved by the Ethics Committee of Iwate Medical University.

Baseline measurements: In the baseline examination, all participants underwent routine anthropometrical measurement, an ECG, blood pressure measurement, and laboratory assessment of cardiovascular risk factors including plasma BNP levels. In addition, a self-administered questionnaire was used to ascertain family history, symptoms, smoking habits, and medical history including the status of drugs prescribed for hypertension, diabetes, hypercholesterolemia, stroke, angina, CHF, and myocardial infarction. Systolic and diastolic blood pressures were determined with an automatic device placed on the right arm of seated subjects who had rested in a sitting position for at least 5 minutes before measurement. The average of 2 such readings was used for statistical analysis. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, and/or use of antihypertensive medication. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m²). Obesity was defined as a BMI ≥ 25 kg/m². Diabetes was ascertained either by patient self-reporting or the presence of a nonfasting glucose concentration ≥ 200 mg/dL or HbA1c value ≥ 6.5%. Renal dysfunction was defined as a serum creatinine level ≥ 1.2 mg/dL.

Plasma BNP measurement: Venous blood samples were drawn from the antecubital vein with the participant resting in a seated position. The samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes. These tubes were stored immediately in an icebox and transported to the central laboratory within 6 hours after collection, and centrifuged at 1,500 g for 10 minutes. After separation, the plasma was stored at -20°C before being assayed for BNP. A noncompetitive immunoradiometric assay based on a 2-site sandwich antibody system (Shionogi & Co., Ltd.) was used to measure BNP levels. The intraassay and interassay coefficients of variation were 5% and 6%, respectively. The lower detection limit of the assay was 0.05 pg/mL.

Reference group: In order to demonstrate the distribution of plasma BNP levels among subjects without evident cardiovascular disease, participants were excluded for the following reasons: any type of ECG abnormality, including major and minor findings, hypertension (see above), history of coronary heart disease (myocardial infarction and/or angina pectoris), diabetes mellitus (see
above), renal dysfunction (see above), and cardiovascular symptoms such as dyspnea on effort, chest pain, chest discomfort, palpitations, and edema. After exclusion of these subjects, a subgroup of 3,410 subjects (mean age, 57 years; 1,056 men and 2,354 women) remained and these were defined as “healthy subjects”.

**Abnormal BNP and risk factors:** Subjects whose plasma BNP levels were greater than the 97.5 percentile point based on the reference group BNP distribution were designated as the “high BNP” group. To examine the relationship between high plasma BNP levels and several clinical characteristics, univariate and multivariate logistic regression analyses were used in the original cohort (n = 8,178). In addition, to elucidate whether this relationship was identical or not in the elderly population, a similar analysis was performed after exclusion of subjects under 65 years of age.

**Statistical analysis:** Data are presented as the mean ± SD. Differences between groups were determined by one-way ANOVA or χ² analysis when appropriate. Plasma BNP was logarithmically transformed for statistical analysis. SPSS software (Chicago, Illinois, USA) was used for statistical analysis. A significant difference was defined as P < 0.05.

**RESULTS**

**All participants:** Plasma BNP levels were found to range widely, with a distribution skewed towards lower levels (data not shown). The minimum value was less than 0.05 pg/mL in both genders, and the maximum values were 1,280 pg/mL in males and 510 pg/mL in females. A small proportion of subjects (8.7% of males and 6.0% of females) had BNP levels below the limit of detection.

Table I presents the coefficients of correlation in simple linear regression analysis between plasma BNP levels and several clinical variables for all participants. The variable related most significantly to plasma BNP level was age in both genders.

**Healthy subjects:** The clinical characteristics of 3,410 healthy subjects (1,056 males and 2,354 females; age, 56.7 ± 11.6 years) are described in Table II. The median value of plasma BNP was 7.6 pg/mL in males and 12.1 pg/mL in females. The median plasma BNP level increased with age in both genders, and was higher in females across all age groups. The 97.5 percentile of BNP levels in the four 10-year age groups (40-49, 50-59, 60-69, and 70-79) were 26.3, 37.2, 64.9, and 72.4 pg/mL in males, and 40.5, 44.5, 61.8, and 77.0 pg/mL in females, respectively.

**Factors contributing to high BNP levels:** Subjects with plasma BNP levels ≥ 97.5 percentile of the gender-age referenced level were designated as the high BNP group (n = 704). Using univariate logistic regression analysis, we tested the associations between each clinical parameter and the high BNP group to evaluate
clinical factors contributing to high plasma BNP levels (Table III). In both genders, hypertension, major ECG abnormalities, and mildly elevated serum creatinine levels were associated with high BNP levels (all, \( P < 0.05 \)). The correlation between high plasma BNP and history of coronary heart disease was evident only in men (\( P < 0.05 \)). Multivariate logistic regression analysis was performed to examine independent clinical factors contributing to high BNP levels (Figure 1). In males, hypertension (\( P < 0.01 \)), major ECG abnormalities\(^{21} \) (\( P < 0.01 \)), history of coronary heart disease (\( P < 0.01 \)), and mildly impaired renal function (\( P < 0.05 \)) were independently associated with high BNP levels. Apart from the history of coronary heart disease, similar trends were found among females (hypertension, \( P < 0.01 \); major ECG abnormalities, \( P < 0.01 \); mildly impaired renal function, \( P < 0.01 \)). No correlation was found between obesity and high plasma BNP levels in either gender.
Age greater than 65 years: We also tested independent clinical parameters contributing to high BNP levels in elderly participants (age ≥ 65). Abnormally high plasma BNP levels (≥ 97.5 percentile of the age-sex referenced level) in healthy elderly subjects were almost the same in both genders (males, 74.0 pg/mL; females, 74.2 pg/mL). We therefore used 74 pg/mL as the cut-off level for abnormally high plasma BNP in elderly subjects. Using multivariate logistic regression, we found that major ECG abnormalities (odds ratio, 7.0; 95% CI, 4.17-11.8; \( P < 0.01 \)), history of coronary heart disease (odds ratio, 2.0; 95% CI, 1.02-4.05; \( P < 0.05 \)), mildly impaired renal function (odds ratio, 1.9; 95% CI, 1.01-3.78; \( P < 0.05 \)), and hypertension (odds ratio, 1.6; 95% CI, 1.15-2.28; \( P < 0.01 \)) were also independently associated with high BNP levels in elderly males. In females, high
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serum creatinine (odds ratio, 5.3; 95% CI, 1.89-15.1; \( P < 0.01 \)), major ECG abnormalities\(^{21}\) (odds ratio, 4.7; 95% CI, 2.74-8.24; \( P < 0.01 \)), and hypertension (odds ratio, 1.5; 95% CI, 1.15-2.28; \( P < 0.01 \)) were independently correlated with high BNP levels.

**Number of CHF risk factors and BNP:** Figure 2 shows the relationship between median plasma BNP levels and the number of risk factors contributing to CHF by gender. Risk factors for CHF were assumed to include advanced age (\( \geq 65 \) years), hypertension, history of coronary heart disease, mildly elevated serum creatinine level, major ECG abnormalities, smoking history, diabetes mellitus, and obesity. The median plasma BNP level in males was 6.7 pg/mL with no CHF risk factors and 33 pg/mL with five or more risk factors. In females, as in males, the accumulation of risk factors increased plasma BNP levels from 12 pg/mL with no risk factors to 28 pg/mL in the group with multiple risk factors.

**DISCUSSION**

To the best of our knowledge, this is the first study to provide cross-sectional data in relation to CHF risk factors and plasma BNP levels in a Japanese community-based population. The main findings of our study can be summarized as follows. First, median plasma BNP levels in a healthy population increase with age and are higher in women. Second, in the adult general population, major ECG abnormalities, hypertension, history of coronary heart disease, and mildly ele-
vated serum creatinine were significant independent variables associated with age- and sex-specific high plasma BNP levels. Third, median plasma BNP levels increase in proportion with the number of established CHF risk factors. The present study therefore demonstrated that, in a general population, subjects with age- and sex-specific reference values for high plasma BNP levels are likely to be at high risk of CHF.

**Importance of identification of CHF high-risk subjects:** Previous reports have suggested that plasma natriuretic peptide measurement is useful for identifying patients with left ventricular systolic dysfunction within a general population and for predicting patients with reduced left ventricular function from among high-risk patients. In addition, plasma natriuretic peptide testing has been reported to have high sensitivity and specificity for the identification of true CHF among patients suspected of having CHF in a primary setting. These observations indicate that plasma BNP measurement may be a helpful aid in the diagnosis of CHF. However, no information is available concerning the utility of plasma natriuretic peptide measurements for screening subjects at high risk for CHF in the Japanese general population.

Since there is a growing clinical interest in subjects at high risk of CHF, as evidenced by its inclusion in a new staging system for CHF, it would seem important to establish a new method for screening subjects with accumulating CHF risk factors or with asymptomatic structural heart disease [stage B in the 2001 ACC/AHA guidelines for CHF] from within large populations. In fact, several reports have shown that effective treatment of hypertension decreases left ventricular hypertrophy, which is known to be a risk factor for CHF. The treatment of asymptomatic patients with diabetes or vascular disease using angiotensin-converting enzyme inhibitors has yielded significant reductions in the prevalence of CHF. Given these considerations, it is important to identify CHF high-risk subjects with asymptomatic structural heart disease who will be prone to develop overt CHF. The present study is the first to demonstrate a relationship between plasma BNP levels and CHF risk factors in the general population, and to suggest that in a screening setting age-and sex-specific referenced high plasma BNP levels may serve as a marker for subjects with an accumulating risk of CHF.

**Age-gender and plasma BNP levels:** Among healthy subjects, plasma BNP levels increase with age and are higher in women than in men. Similar trends have been observed previously. These findings suggest that we should include consideration of the effects of age and gender in the interpretation of plasma BNP results. There are several possible explanations for the observed difference in median plasma BNP levels between genders. In healthy subjects, median plasma BNP values were higher in women in all age groups. Redfield, et al reported that there
was a relationship between hormone replacement therapy and increased plasma BNP levels in women,\textsuperscript{16)} suggesting an influence of estrogen. Other investigators have reported that women had significantly higher left ventricular end-systolic elastance and lower passive diastolic compliance compared with age-adjusted men.\textsuperscript{32,33)} These left ventricular biomechanical characteristics in women may stimulate BNP production from the heart,\textsuperscript{34)} giving rise to the difference in plasma BNP levels between the sexes.

**Clinical implications:** Echocardiography may be one of the most important techniques for screening for asymptomatic structural heart disease with high CHF risk. However, given the cost and resources necessary to implement standard echocardiographic screening in the general population, it would not be practical to employ this technology. If a reliable biomarker could be established, early intervention would become possible. This would make it possible to limit the progression to overt CHF, resulting in a significant improvement in outcomes for CHF. Plasma BNP measurement is relatively inexpensive and BNP can easily be assayed without radioisotope labeling. In addition, plasma BNP has been reported to be relatively stable after storage at room temperature for several hours.\textsuperscript{35)} It follows, therefore, that plasma BNP testing may be a candidate biomarker for screening for subjects at high risk of CHF.

**Limitations:** The present study may have failed to detect some subjects with asymptomatic cardiac disorders as echocardiography was not performed in the baseline examination, and the reference values might be biased. However, to obtain the reference level we carefully excluded participants with hypertension, any type of ECG abnormality, previous myocardial infarction, mildly impaired renal function, cardiovascular medications, and cardiac symptoms. It therefore seems unlikely that this bias may have significantly affected our findings.

Because our population was a sample from a multiphasic health checkup, the data presented here need to be interpreted with some caution due to selection bias. However, the present study covered more than 20% of the age-matched population in the area. Moreover, there were very few differences in the frequency of hypertension and history of stroke, and in the distribution of plasma total cholesterol, random blood glucose levels, and body mass index between this cohort and data from a recent national health survey conducted in a randomly selected adult population.\textsuperscript{36)} These findings suggest that the selection bias in our study may have been limited. We could not find any association between high plasma BNP levels and history of coronary heart disease in females. This may be explained by the small number of female subjects having a history of coronary heart disease (females: 1.0%, males: 2.4%), especially myocardial infarction. This would mean that the statistical power to detect a significant association in this context may have been limited.
In conclusion, this community-based study has demonstrated that age- and sex-specific reference values for high plasma BNP levels indicate an accumulation of CHF risk factors. This suggests that plasma BNP measurement may be a useful screening test for identifying individuals at high risk of CHF within a Japanese general population.

REFERENCES