N-Terminal Pro-Brain Natriuretic Peptide and Risk of Cardiovascular Events in a Japanese Community

The Hisayama Study

Yasufumi Doi, Toshiharu Ninomiya, Jun Hata, Yoichiro Hirakawa, Naoko Mukai, Fumie Ikeda, Masayo Fukuhara, Masanori Iwase, Yutaka Kiyohara

Objective—Few studies have examined the association between natriuretic peptides and the incidence of cardiovascular disease (CVD) in Asian populations.

Methods and Results—A total of 3104 community-dwelling Japanese individuals aged ≥40 years without history of CVD were followed up for 5 years. A total of 127 CVD events were identified. The age- and sex-adjusted incidence of CVD increased with increasing N-terminal pro-brain natriuretic peptide (NT-proBNP) levels (<55, 55–124, 125–399, and ≥400 pg/mL) at baseline and was significantly higher even in subjects with a modest increase. This association remained robust even after adjustment for other potential risk factors (55–124 pg/mL: multivariate-adjusted hazard ratio 1.85 [95% CI 1.07–3.18], P=0.03; 125–399 pg/mL: 2.98 [95% CI 1.65–5.39], P<0.001; ≥400 pg/mL: 4.54 [95% CI 2.22–9.29], P<0.001). The multivariate-adjusted hazard ratios for the development of total CVD and its subtypes, coronary heart disease and stroke, were significantly increased by a 1 SD increment of the log NT-proBNP concentrations and were nearly equal among CVD subtypes. Similar findings were observed for stroke subtypes of ischemic stroke and intracerebral hemorrhage but not subarachnoid hemorrhage. The effects of the 1 SD increment in log NT-proBNP values were comparable in subjects with and without other cardiovascular risk factors, except for sex. The area under the receiver operating characteristic curve was significantly (P=0.006) increased by adding NT-proBNP values to the model including other potential risk factors.

Conclusion—Elevated NT-proBNP levels were shown to be a significant risk factor for the development of CVD and its subtypes in a general Japanese population, independently of other cardiovascular risk factors. (Arterioscler Thromb Vasc Biol. 2011;31:2997-3003.)

Key Words: coronary artery disease ■ epidemiology ■ risk factors ■ stroke ■ cohort study

B-type natriuretic peptide (BNP) is a cardiac hormone secreted from the myocardium in response to increased ventricular stretch and wall tension. The precursor of BNP is split equimolarly into a biologically active peptide and a more stable N-terminal fragment (N-terminal pro-BNP [NT-proBNP]).1-2 Measurement of circulating BNP or NT-proBNP levels has been recommended in the diagnosis and prognosis of patients with symptoms of left ventricular dysfunction3 and for stratification of prognosis in patients with acute coronary syndromes.4,5 Several prospective studies of community-dwelling persons have focused on the association between BNP/NT-proBNP and the risk of cardiovascular disease (CVD), particularly in white populations.6-11 However, it is not certain to what extent these findings apply to general Asian populations. In addition, to the best of our knowledge, no studies have evaluated the association between BNP/NT-proBNP levels and hemorrhagic stroke, which has pathophysiological mechanisms different from those of thrombotic diseases.

The objective of the current study was to examine the associations between NT-proBNP levels at baseline and the development of CVD and its subtypes in a general Japanese population, taking into account comprehensive confounders.

Methods

Study Population
A population-based prospective study of CVD and its risk factors has been under way since 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area on Japan’s Kyushu Island. The age, occupational distributions, and nutritional intake of the population were almost identical to those of the general Japanese population, based on data from the national census and nutrition survey.12 In 2002, a baseline survey for the present study was performed in the town. A detailed description of this survey was published previously.13 Briefly, of all residents aged ≥40 years, 3328 underwent...
examination (participation rate, 77.6%). A total of 224 individuals were subsequently excluded from the study; among these, 30 subjects did not consent to participate in the study, 190 had a history of CVD, and 4 had an insufficient quantity of stored sera for NT-proBNP measurement. Overall, 3104 individuals (1303 men and 1801 women) were enrolled in the study.

Follow-Up Survey
The subjects were followed up prospectively for 5 years, from 2002 to 2007, by repeated health examinations. The health status was checked yearly by mail or telephone for subjects who did not undergo a regular examination or who had moved away from town. We also established a daily monitoring system among the study team, local physicians, and members of the town’s Health and Welfare Office. Using this system, we gathered information on new CVD events, including suspected cases. When coronary heart disease or stroke occurred or was suspected, physicians in the study team examined the subject and evaluated his or her detailed clinical information. The clinical diagnosis of coronary heart disease or stroke was based on the patient’s history, physical and neurological examinations, and ancillary laboratory examinations. Additionally, when a subject died, an autopsy was performed at the Department of Pathology of Kyushu University. During the follow-up period, no subject was lost to follow-up, and 192 subjects died, of whom 129 (67.2%) underwent autopsy.

Definition of End Points
The outcomes of the present analysis were incidence and mortality of CVD. Total CVD was diagnosed as the development of coronary heart disease and stroke, and CVD death was defined as I00 to I99 of International Classification of Diseases-10.

The diagnosis and classification of stroke were determined on the basis of clinical information, including brain computed tomography and magnetic resonance imaging, cerebral angiography, echoradiography, carotid duplex imaging, and autopsy findings.14 In principle, stroke was defined as a sudden onset of nonconvulsive and focal neurological deficit persisting for >24 hours. Stroke was further divided into ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage based on the Classification of Cerebrovascular Disease III proposed by the National Institute of Neurological Disorders and Stroke.15

The criteria for the diagnosis of coronary heart disease included acute and silent myocardial infarction, sudden cardiac death within 1 hour after the onset of acute illness, coronary artery angioplasty, and bypass grafting. The diagnosis of myocardial infarction was based on detailed clinical information and at least 2 of the following findings: typical clinical symptoms; ECG evidence of myocardial infarction; elevated cardiac enzymes; and morphological findings, including echocardiographic, scintigraphic, or angiographic abnormalities compatible with myocardial injury, and autopsy findings. Silent myocardial infarction was defined as myocardial scarring without any historical indication of clinical symptoms or abnormal cardiac enzyme changes.

During the follow-up, 127 first-ever CVD events (77 men and 50 women) and 48 CVD deaths occurred. Among the CVD events, there were 49 cases of coronary heart disease (36 cases of myocardial infarction, 8 of coronary artery angioplasty, 2 of coronary artery bypass grafting, and 3 of sudden cardiac death) and 83 of stroke (54 cases of ischemic stroke, 19 of intracerebral hemorrhage, 9 of subarachnoid hemorrhage, and 1 of unclassified stroke).

Clinical Evaluation and Laboratory Measurement
At the screening examination, a portion of a serum specimen was stored at −80°C until it was used for the measurement of NT-proBNP concentrations in 2009. NT-proBNP levels were measured using a second-generation commercial kit, the Elecsys proBNP Immunoassay,16 on an Elecsys 2010 platform.

Serum creatinine was measured by the enzymatic method using a fresh blood sample. The estimated glomerular filtration rate (eGFR) was calculated using the following modified equation of the Modification of Diet in Renal Disease Study for Japanese:17

\[
eGFR (\text{mL/min per 1.73 m}^2) = \frac{0.808 \times 175 \times (\text{serum creatinine [mg/dL]} \times 2.1)^{1.154} \times \text{age [years]}^{-0.203}}{0.741 (\text{Japanese coefficient}) \times (0.742 \text{ if female})}.
\]

Urine creatinine and albumin were measured using a spot urine sample by the turbidimetric immunoassay method. The urine albumin-creatinine ratio (mg/g) was calculated by dividing the urinary albumin values by the urinary creatinine concentrations. Chronic kidney disease was defined as an eGFR of <60 mL/min per 1.73 m² or a urine albumin-creatinine ratio of ≥30 mg/g according to the National Kidney Foundation’s Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines.18 Plasma glucose concentrations were determined by the glucose-oxidase method. Diabetes was defined as fasting glucose concentrations ≥7.0 mmol/L, 2-hour postload or postprandial glucose concentrations ≥11.1 mmol/L, or taking antidiabetic medications. Total and high-density lipoprotein cholesterol levels were determined enzymatically, and hypercholesterolemia was defined as a total cholesterol level of ≥5.69 mmol/L.

Blood pressure was obtained 3 times using an automated sphygmomanometer (BP-203RV III, Colin, Tokyo, Japan) with the subject in a sitting position; the average of the 3 measurements was used in the present analysis. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or current treatment with antihypertensive agents. ECG abnormalities were defined as left ventricular hypertrophy (Minnesota Code 3-1), ST depression (4-1, 4-2, or 4-3), or atrial fibrillation (8-3).

Height and weight were measured with the subject wearing light clothes without shoes, and body mass index (BMI) (kg/m²) was calculated. Obesity was defined as a BMI level of ≥25 kg/m². Each participant completed a self-administered questionnaire covering medical history, smoking habits, alcohol intake, and exercise. Smoking habits and alcohol intake were classified as either current habitual use or not. Those subjects who were engaged in sports or other forms of exertion ≥3 times a week during their leisure time made up a regular exercise group.

Statistical Analysis
NT-proBNP levels were divided into 4 categories: <55, 55 to 124, 125 to 399, and ≥400 pg/mL according to the prior reports.3,19,20 Age- and sex-adjusted mean values for possible risk factors were calculated by analysis of covariance, and their trends across NT-proBNP levels were tested by multiple regression analysis. Frequencies of risk factors were adjusted for age and sex by the direct method and were examined for trends by the Cochran-Mantel-Haenszel test. The incidence of CVD was calculated by the person-year method and was adjusted for age and sex by the direct method using 10-year age groupings. The age- and sex-adjusted or multivariate-adjusted hazard ratios (HRs) and their 95% CIs were calculated using Cox proportional hazards model. The linear trends of HRs were examined for trends by the Cochran-Mantel-Haenszel test. The incidence of CVD was calculated by the person-year method and was adjusted for age and sex by the direct method using 10-year age groupings. The age- and sex-adjusted or multivariate-adjusted hazard ratios (HRs) and their 95% CIs were calculated using Cox proportional hazards model. The linear trends of HRs across NT-proBNP levels were also tested using the Cox proportional hazards model. Comparisons of the effects of increased NT-proBNP values between participants with and without other cardiovascular risk factors were made by adding an interaction term to the statistical model. To compare the accuracy of risk assessment for CVD development between the models adjusted for potential risk factors with and without NT-proBNP values, receiver operating characteristic (ROC) curves for the model were plotted. The consistency in the area under the ROC curve between the models was estimated using the method of DeLong et al.21 All analyses were performed using the SAS software package version 9.2 (SAS Institute Inc, Cary, NC). Values of P<0.05 were considered statistically significant in all analyses.

Ethical Considerations
This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Research, and written informed consent was obtained from all the participants.
Results

The baseline characteristics of the study population according to the 4 categories of NT-proBNP values are summarized in Table 1. The mean values of age and systolic blood pressure and the frequencies of hypertension, ECG abnormalities, and chronic kidney disease increased with increasing NT-proBNP levels, whereas the mean values of eGFR, BMI, and total cholesterol and the aggregation of each frequency of men, obesity, hypercholesterolemia, and regular exercise declined significantly with rising NT-proBNP levels.

Table 2 shows the age- and sex-adjusted incidence of CVD according to NT-proBNP levels. A significant association was observed between NT-proBNP levels and the incidence of total CVD. In regard to subtypes of CVD, the incidence of coronary heart disease and stroke increased significantly as the NT-proBNP levels increased. Similar findings were observed in stroke subtypes of ischemic stroke and intracerebral hemorrhage, but not subarachnoid hemorrhage. The HR for the development of total CVD increased with increasing NT-proBNP levels and was significantly higher even in subjects with NT-proBNP levels of 55 to 124 pg/mL compared with those with NT-proBNP levels of <55 pg/mL after adjustment for age, sex, systolic blood pressure, ECG abnormalities, eGFR, BMI, diabetes, total and high-density lipoprotein cholesterol levels, smoking habits, alcohol intake, and regular exercise. Furthermore, when estimating the age- and sex-adjusted and multivariate-adjusted HRs for a 1 SD increment in log-transformed NT-proBNP concentrations, we found significant upward trends for the development of total CVD and its subtypes (ie, coronary heart disease and stroke, including ischemic stroke and intracerebral hemorrhage), and the magnitude of the influence of the 1 SD increment in log NT-proBNP concentrations was almost equal among CVD subtypes (Table 2).

In the same way as the CVD incidence, the age- and sex-adjusted mortality from CVD increased with increasing NT-proBNP levels and was significantly higher in subjects with NT-proBNP levels of 125 to 399 pg/mL compared with those with the lowest NT-proBNP levels (Table 2). This association remained unchanged even after adjustment for the confounding factors mentioned above.

The age- and sex-adjusted HRs for the development of total CVD owing to a 1 SD increment in log NT-proBNP concentrations, in subjects with and without other cardiovascular risk factors, are shown in Figure 1. Comparable effects of a 1 SD increment in log NT-proBNP concentrations on the risk of total CVD were observed in subjects aged 40 to 64 years and those aged ≥65 years (P for heterogeneity = 0.82). A sex difference in the influence of a 1 SD increment in log NT-proBNP concentrations on the incidence of CVD was identified, although the association between log NT-proBNP concentrations and the incidence of CVD was statically
Table 2. Adjusted Incidences, Mortalities, and Hazard Ratios of Cardiovascular Disease and Its Subtypes According to NT-proBNP Levels, 2002 to 2007

<table>
<thead>
<tr>
<th>NT-proBNP levels (pg/ml)</th>
<th>Total cardiovascular disease incidence</th>
<th>P for Trend (Across Categories)</th>
<th>Continuous Log Scale*</th>
<th>P for Trend (Continuous)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=1606)</td>
<td>(n=915)</td>
<td>(n=444)</td>
<td>(n=139)</td>
<td></td>
</tr>
<tr>
<td>&lt;55</td>
<td>25/8304</td>
<td>37/4641</td>
<td>41/2082</td>
<td>24/542</td>
</tr>
<tr>
<td>Age- and sex-adjusted†</td>
<td>4.0</td>
<td>8.6</td>
<td>14.2</td>
<td>38.6</td>
</tr>
<tr>
<td>Multivariable-adjusted HR (95% CI)</td>
<td>1 (reference)</td>
<td>1.89 (1.10–3.24)</td>
<td>3.26 (1.83–5.80)</td>
<td>4.99 (2.55–9.74)</td>
</tr>
</tbody>
</table>

Coronary heart disease

| No. of events/person-y | 9/8339                              | 16/4691                         | 15/2150               | 9/574                   |
| Age- and sex-adjusted†   | 1.8                                  | 3.6                              | 4.0                   | 6.0                     |
| Multivariable-adjusted HR (95% CI) | 1 (reference) | 2.11 (0.88–5.05) | 2.98 (1.16–7.67) | 4.18 (1.41–12.42) | 0.007  | 1.63 (1.25–2.13) | <0.001 |

Total stroke

| No. of events/person-y | 16/8318                            | 22/4656                         | 30/2095               | 15/552                  |
| Age- and sex-adjusted†   | 2.2                                  | 5.2                              | 10.9                  | 31.9                    |
| Multivariable-adjusted HR (95% CI) | 1 (reference) | 1.77 (0.90–3.50) | 3.81 (1.88–7.74) | 5.20 (2.24–12.06) | <0.001 | 1.75 (1.43–2.14) | <0.001 |

Total cardiovascular disease mortality

| No. of events/person-y | 2/8353                             | 12/4709                         | 16/2163               | 18/584                  |
| Age- and sex mortality†   | 0.5                                  | 2.4                              | 6.2                   | 12.3                    |
| Multivariable-adjusted HR (95% CI) | 1 (reference) | 3.91 (0.85–18.02) | 5.25 (1.11–24.83) | 12.70 (2.57–62.72) | <0.001 | 1.93 (1.46–2.56) | <0.001 |

Adjustment was made for age, sex, systolic blood pressure, electrocardiogram abnormalities, estimated glomerular filtration rate, body mass index, diabetes, total and high density lipoprotein cholesterol levels, smoking habits, alcohol intake, and regular exercise. NT-proBNP indicates N-terminal pro-brain natriuretic peptide; HR, hazard ratio.

*HR per 1 SD increase of log NT-proBNP.
†Per 1000 person-y.

significant in both sexes. There were no clear differences in the effects of NT-proBNP in subjects with and without other cardiovascular risk factors, such as hypertension, chronic kidney disease, obesity, diabetes, hypercholesterolemia, and smoking (all probability values for heterogeneity >0.05).

To evaluate the influence of NT-proBNP levels on the accuracy of CVD risk assessment, we compared the area under the ROC curve between models with and without NT-proBNP values (Figure 2). The area under the ROC curve was significantly increased by adding NT-proBNP values to the model including the potential risk factors mentioned above (from 0.820 to 0.841; P for difference in the area=0.006). The same was true for the respective CVD subtypes—ie, coronary heart disease and stroke (data not shown).

To compare the ability of NT-proBNP to predict future CVD with other risk factors, we estimated the areas under the ROC curves, adding continuous values of risk factors to the age- and sex-adjusted model. As a result, the area under the ROC curve was significantly larger for NT-proBNP (0.816) than for other risk factors, namely, systolic blood pressure (0.795), eGFR (0.784), BMI (0.782), total cholesterol (0.786), and high-density lipoprotein cholesterol (0.783) (all P<0.05).
In a prospective study of a general Japanese population, we clearly demonstrated that the risk for the development of CVD and its subtypes increased with increasing NT-proBNP levels and was significantly higher even in subjects with a modest increase in NT-proBNP. This association remained robust even after controlling for other confounding factors. The magnitude of the influence of NT-proBNP was nearly equal among CVD subtypes and larger than other risk factors. These findings suggest that high NT-proBNP levels are an independent and strong risk factor for the development of various types of CVD.

Several cohort studies have indicated that elevated BNP/NT-proBNP levels increased the risk of total CVD, coronary heart disease, and stroke. However, very few prospective studies have provided evidence of associations between the natriuretic peptide levels and CVD in Asian populations. One cohort study of a Japanese population showed an association between increased BNP levels and the risk of developing ischemic stroke. Another clinical observational study in Japan also revealed a significant influence of elevated log NT-proBNP concentrations was almost equal among CVD subtypes: namely, coronary heart disease, ischemic stroke, and intracerebral hemorrhage. In white population–based studies, a 1 SD increase in log NT-proBNP concentrations was associated with a 35% to 92% increase in the risk of major CVD events. These findings imply that the measurement of NT-proBNP values is valuable for identifying individuals at high risk of CVD independent of ethnicity.

In our study, NT-proBNP levels were inversely related to obesity and hypercholesterolemia and positively related to age and blood pressure levels and the prevalence of chronic kidney disease. Nevertheless, the inclusion of these factors in the model did not attenuate the overall association between NT-proBNP levels and total CVD, and adding NT-proBNP values to potential risk factors significantly increased the area under the ROC curve. Furthermore, our study revealed no interactions between the various risk factors and NT-proBNP values, with the exception of sex. These findings imply that NT-proBNP is a novel and universal risk factor for various CVDs.
types of CVD and may improve the risk prediction of future CVD events in general populations beyond the estimation afforded by classical risk factors.

The mechanisms underlying the association between natriuretic peptides and the risks of CVDs are still unknown. A cross-sectional study found that high NT-proBNP levels were independently associated with higher coronary artery calcium scores as evaluated by electron beam computed tomography in subjects without heart failure or renal dysfunction. This fact raises the possibility that BNP/NT-proBNP levels are correlated with the degree of systemic atherosclerosis. Because elevated natriuretic peptides reflect increased ventricular stretch from volume as well as pressure overloads, subjects with these overloads might have vascular stretch and wall tension, which could contribute to the development of CVD. However, NT-proBNP levels are considered a surrogate marker because medication with BNP has an effect on relaxation, not on tension, of the human arteries. To date, no specific pathological role of natriuretic peptides has been identified in inflammation, oxidative stress, or abnormalities in coagulation and fibrinolytic pathways, which are considered to be the major pathological mechanisms involved in the atherosclerotic process. Additional studies are needed to reveal the mechanisms of the association between NT-proBNP levels and vascular damage.

The strengths of our study include its longitudinal population-based design, low selection bias, perfect follow-up of subjects, and accuracy of diagnosis of CVD subtypes. One limitation of our study is that the evaluation of NT-proBNP values was based on a single measurement at baseline, as is the case in most epidemiological studies. During the follow-up, the levels were changed because of modifications in lifestyle or medication, and misclassification of NT-proBNP levels was possible. This could have weakened the association found in this study, biasing the results toward the null hypothesis. Therefore, the true association may be stronger than that shown in our study.

In conclusion, the present analysis clearly showed that elevated NT-proBNP levels were a novel risk factor for CVD and its subtypes—ie, coronary heart disease and stroke—in a general population of Japanese. This study also demonstrated the potential applicability of NT-proBNP measurement to epidemiological studies; such an approach may be suitable for large-scale screening programs to evaluate the risk of CVD, including coronary heart disease and stroke.

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