Plasma B-Type Natriuretic Peptide Is Useful for Cardiovascular Risk Assessment in Community-Based Diabetes Subjects

Comparison With Albuminuria

Masayuki ONODERA,¹ MD, Motoyuki NAKAMURA,¹ MD, Fumitaka TANAKA,¹ MD, Tomohiro TAKAHASHI,¹ MD, Shinji MAKITA,¹ MD, Takenori ISHISONE,¹ MD, Yasuhiro ISHIBASHI,¹ MD, Kazuyoshi ITAI,² PhD, Toshiyuki ONODA,² MD, Masaki OHSAWA,² MD, Kozo TANNO,² MD, Kiyomi SAKATA,² MD, Shinichi OMAMA,³ MD, Kuniaki OGASAWARA,³ MD, Akira OGAWA,³ MD, Toru KURIBAYASHI,⁴ PhD, Kentaro SAKAMAKI,⁶ MPH, and Akira OKAYAMA,⁵ MD

SUMMARY

Diabetes mellitus (DM) is a strong risk factor for cardiovascular (CV) disease. Plasma B-type natriuretic peptide (BNP) levels are elevated in various types of cardiac diseases. Increased plasma BNP levels have been reported to be associated with CV risk in apparently healthy individuals. However, no studies have yet examined the specific value of plasma BNP for predicting CV incidence in unselected DM subjects in a community-based population.

In a community-based DM cohort ($n = 1,059$, mean = 66 years), baseline BNP levels were determined, and CV events were followed and captured prospectively. The cohort was divided by plasma BNP quintiles. The Cox proportional hazard model was used to determine the relative hazard ratios (HR) among the quintiles. In addition, the effects of adding the plasma BNP or urine albumin-to-creatinine ratio (UACR) to an established CV risk scoring model was examined by calculating the area under the receiver operating characteristic (ROC) curve (AUC).

During the 5.7 year follow-up period, CV events were identified in 65 of the DM cohort. There was a significant association between plasma BNP levels and CV event rate ($P < 0.001$). HR was significantly increased in the highest quintile compared to the lowest (HR = 4.38; 95%CI 1.69 -11.84). The AUC generated from ROC analysis of the Framingham risk score for predicting general CV events was improved by adding BNP testing (from 0.66 to 0.74; $P = 0.05$), but not by adding UACR (from 0.66 to 0.67; $P = 0.49$).

In a community sample of people with DM, plasma BNP levels above the 80 percentile are directly associated with CV risk, and measurement of plasma BNP alone or in conjunction with an established CV risk score is of value in predicting CV events in these subjects. (Int Heart J 2012; 53: 176-181)

Key words: Longitudinal study, Heart failure, Stroke, Myocardial infarction

It is well accepted that patients with diabetes mellitus (DM) are at risk for cardiovascular (CV) diseases such as coronary artery disease, left ventricular hypertrophy and dysfunction, heart failure, and stroke. The worldwide prevalence of DM has risen dramatically over the past several decades. Although the need for individualized and timely risk assessment has been emphasized, some DM subjects remain free of cardiovascular (CV) disease while others experience serious CV complications. Several studies have suggested that aggressive pharmacological and nonpharmacological interventions improve CV prognosis in patients with DM.^{1,2)} It may therefore be realistic to select DM subjects at relatively high risk for CV disease, and then provide intensive risk factor modification to prevent the development of CV events.

Many biomarkers, such as microalbuminuria including

urine albumin-to-creatinine ratio (UACR), blood pressure, lipid parameters, and renal function have been put forward as potential indicators of elevated CV risk in individuals with DM. To some extent, however, these variables are of limited use as they are merely mediators for long-term CV injury and are not direct markers of cardiac and vascular abnormality. The natriuretic peptide family, including B-type natriuretic peptide (BNP) and its N-terminal fragment (NT-proBNP), are released from the heart in response to increased intracardiac pressure, cardiac pump dysfunction, hypertensive ventricular hypertrophy, and myocardial ischemia. BNP/NT-proBNP may thus be a direct biomarker for clinical or subclinical cardiac abnormality. In fact, a few community-based studies have shown that increased levels of BNP/NT-proBNP were associated with CV risk and mortality independent of established CV risk markers.^{3,4)}

From the Departments of ¹ Internal Medicine, ² Preventive Medicine, and ³ Neurosurgery, Iwate Medical University, ⁴ Department of Health and Physical Education, Iwate University, Iwate, and ⁵ The First Anti-tuberculosis Association, ⁶ Department of Biostatistics, School of Public Health, The University of Tokyo, Tokyo, Japan.

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Address for correspondence: Motoyuki Nakamura, MD, Division of Cardiovascular Medicine, Department of Internal Medicine, Iwate Medical University, 19-1 Uchi-marui, Morioka, Iwate 020-8505, Japan.

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Several previous studies have shown that, in selected patients with DM treated by diabetologists in teaching hospitals, increased levels of plasma BNP/NT-proBNP were related to risk of all-cause mortality and CV death.⁵⁻⁹⁾ Although such a simple risk screening approach in the DM population may be more helpful for general practitioners or health care providers as a signal for commencement of evidence-based interventions for DM, the specific value of plasma BNP for predicting increased CV risk in unselected DM subjects in a community-based population has yet to be established. Moreover, no studies have examined the effects of adding plasma BNP testing to a traditional CV risk prediction model in these subjects.

The aim of the present study was to determine the level of plasma BNP which marked an increased incidence of CV events in unselected community-based individuals with DM. Further, the effects of adding BNP or UACR to an established CV risk scoring system were examined in terms of an increase in the area under the curve (AUC) generated from a receiver-operating-characteristic (ROC) analysis.

METHODS

Study subjects: Participants in the present study were part of the Iwate-KENCO study, and the details of recruitment and measurements in the cohort have been provided in previous reports.^{10,11)} In brief, the original cohort ($n = 26,469$) was recruited from the population living in Ninohe, Kuji, and Miyako districts of northern Iwate prefecture, Japan, during 2002-2004. Of the original cohort living in Ninohe and Kuji districts ($n = 15,927$), 15,394 subjects (97%) underwent BNP measurement (BNP cohort: men 5,288; women 10,106). In the BNP cohort, DM individuals were selected by one or more of the following criteria; 1) casual blood glucose level ≥ 200 mg/dL or fasting blood glucose ≥ 126 mg/dL, 2) use of antidiabetic agents including insulin, and 3) glycosylated hemoglobin (HbA1c) $\geq 6.5\%$ (NGPS). Subjects were excluded from the present analysis for the following reasons: age under 40 or over 90 years; missing data on serum creatinine levels, body mass index (BMI), ECG tracing, and blood pressure data. The final statistical analysis for the DM cohort was therefore performed on 1,059 subjects (men 509; women 550; mean age, 66.1 years). The study protocol was approved by the university ethics committee and by local institutional review committees. All participants gave written informed consent.

Measurements: All subjects used a self-report questionnaire to confirm their medical history, including status (yes/no) of prescribed drugs for hypertension, diabetes, and hyperlipidemia. Smoking status (current, past, or nonsmoker) was also assessed by questionnaire. Systemic blood pressure in a sitting position after at least 5 minutes rest was measured by experienced research staff using an automatic digital device. The measurement was performed twice, with the mean value used for statistical analysis. Casual blood samples were taken from upper arm veins with subjects in a seated position. Samples were collected into vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) or a serum separator gel. Blood glucose was determined using an enzymatic method. Glycosylated hemoglobin (HbA1c) was measured quantitatively using values established by the Japan Diabetes Society (JDS) with an HPLC method (Tosoh, Japan). The value for HbA1c (%) in

this paper was presented as the NGSP equivalent value (%) calculated by the formula $\text{HbA1c} = \text{HbA1c (JDS)} + 0.4$.¹²⁾ Enzymatic methods were used to measure serum total cholesterol levels and serum creatinine. The eGFR was calculated using the equation $[\text{eGFR (mL/minute/1.73 m}^2\text{)} = 194 \times \text{Cr}^{-1.094} \times \text{Age}^{-0.287}]$ taken from the Modification Diet in Renal Disease Study (MDRD) for the Japanese population.¹³⁾ Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of antihypertensive medication. Hyperlipidemia was defined by a total cholesterol concentration ≥ 240 mg/dL and/or use of lipid-lowering agents. BMI was calculated as weight (kg) divided by the square of height (m). The established CV risk score was calculated using the Framingham 10-year risk score (FRS) including age, sex-specific cholesterol, HDL cholesterol, systolic blood pressure, diabetes, and cigarette smoking.¹⁴⁾

BNP and UACR: For BNP measurement a separate 2 mL venous blood sample was collected into a test tube containing EDTA acid sodium. Tubes were stored immediately after sampling in an icebox and were transported to the laboratory. They were then centrifuged at 1500 g for 10 minutes. After separation, the plasma samples were stored frozen at -20°C , and BNP levels were measured by direct radioimmunoassay using monoclonal antibodies specific for human BNP (Shionogi, Japan). Cross-reactivity of the antibody was 100% for human BNP and 0.001% for human atrial natriuretic peptide. Intra- and interassay coefficients of variation were 5% and 6%, respectively. The lower detection limit of the assay was 0.05 pg/mL.

A spot urine was also collected, cooled and transported to the laboratory during each afternoon and analyzed on the same day. Urine albumin was assessed quantitatively by an immunonephelometric method (N-antiserum albumin, Dade Behring) and urine creatinine was measured quantitatively by an enzymatic colorimetric test. Urine albumin concentration (mg/dL) was divided by urine creatinine concentration and expressed as UACR (mg/g). The accuracy of this ratio to compare with the 24-hour urine sample had been demonstrated in previous papers.^{15,16)} The sensitivity limit for albumin was 6 mg/L. Interassay and intraassay CV were both within 5%.

Outcome: A follow-up survey assessing the incidence of CV events was carried out after the baseline study (from 2002 to 2004). We defined CV events as a composite of congestive heart failure, stroke, and myocardial infarction. Hospital admissions for congestive heart failure and myocardial infarction in the cohort were identified by accessing data from the Northern Iwate Heart Disease Registry Consortium. Heart failure was defined by Framingham criteria,¹⁷⁾ and registration of myocardial infarction was based on criteria used in the MONICA study.¹⁸⁾ Stroke events were identified by accessing the Prefecture Stroke Registration Program conducted by the Iwate Medical Association. Stroke diagnostic criteria in this registry are based on those published by the World Health Organization and defined as the sudden onset of neurological symptoms.¹⁹⁾ These registrations were initially performed by attending physicians at each hospital using a registration card after admission. Furthermore, to ensure complete capture of all registrations, researchers visited and checked medical charts and/or discharge summaries at referral hospitals within the study district. All-cause deaths and migration were confirmed by the official resident registration data issued by the local government offices (October 2009).

Statistical analysis: Continuous variables are shown as mean $\pm$ SD. To test the relationships between BNP and UACR, log-transformation of each biomarker was performed. DM subjects were divided into quintiles according to their baseline levels of plasma BNP and UACR. To compare results among quintiles, ANOVA or chi-square test was used as appropriate. Follow-up for CV events was carried out after the baseline study through to March 2009. For analyses of CV incidence, person-years were censored at the date of CV events, the date of migration from the study area, the date of death, or the end of the follow-up period, whichever came first. Survival from entry into the study was estimated using the Kaplan-Meier method, followed by a trend test (log rank). Using a Cox proportional hazards regression model, sex-age adjusted hazard ratios (HRs) for these biomarkers with CV events were assessed. In addition, a multi-variable proportional-hazard regression analysis was performed to assess the threshold level for increased risk of CV events (adjustment factors; sex, age, systolic BP, diastolic BP, BMI, HbA1c, CV history, smoking, hyperlipidemia, atrial fibrillation, estimated GFR, use of antihypertensive drugs, and use of anti-DM drugs). To compare the additive effects of plasma BNP or UACR in conjunction with the FRS in terms of the

overall diagnostic accuracy of the tests, ROC curves were constructed and AUC and 95% confidence intervals (CI) for each ROC curve were calculated. The statistical analysis was performed using SPSS (Version 11.0.1J) or MedCalc (Version 9.6.0.0). A significant difference was defined as $P < 0.05$.

RESULTS

Several clinical characteristics of the DM cohort are shown in Table I. The number of DM cases was 1,059. Mean age was 66 years old, and the mean HbA1c level was 7.2%. In this DM cohort, prevalence rates for hypertension, obesity (BMI ≥ 25.0), current smoking, and atrial fibrillation were 57.2%, 9.3%, 16.6%, and 3.0%, respectively. The median level of plasma BNP was 16.3 pg/mL (men = 15.3, women = 18.2 pg/mL; $P = 0.584$). Among the BNP quintiles, mean age and percentage of using diabetic agents and antihypertensive drugs were higher in the highest quintiles than the lower quintiles. The percentage of subjects using lipid-lowering agents did not differ significantly among the quintiles. Plasma BNP levels were significantly correlated with eGFR ($r = -0.222$, $P <$

Table I. Baseline Characteristics According to Quintiles of Plasma BNP and UACR in DM Cohort

	Total (n = 1059)	BNP					P^*
		Q1 (n = 211)	Q2 (n = 213)	Q3 (n = 211)	Q4 (n = 212)	Q5 (n = 212)	
Inter quartile level	-	1.0-4.3	7.2-8.6	14.5-16.3	24.7-28.3	46.5-62.3	
Age (years)	66.1 $\pm$ 9.1	60.4 $\pm$ 9.4	63.4 $\pm$ 9.1	66.6 $\pm$ 8.1	68.5 $\pm$ 7.2	71.5 $\pm$ 7.1	< 0.001
Male/Female	509/550	103/108	117/96	103/108	90/122	96/116	0.114
BMI (kg/m ²)	25.2 $\pm$ 3.7	25.6 $\pm$ 3.8	25.1 $\pm$ 3.3	25.2 $\pm$ 3.7	25.3 $\pm$ 3.8	24.6 $\pm$ 3.7	0.072
Systolic BP (mmHg)	133 $\pm$ 19	131 $\pm$ 18	130 $\pm$ 20	132 $\pm$ 19	135 $\pm$ 19	137 $\pm$ 20	0.001
Diastolic BP (mmHg)	76 $\pm$ 10	78 $\pm$ 10	77 $\pm$ 11	76 $\pm$ 10	76 $\pm$ 11	75 $\pm$ 11	0.054
Hypertension (%)	57.2	45.0	50.0	56.9	64.6	69.8	< 0.001
drugs (%)	39.4	25.6	33.3	41.2	44.8	51.9	< 0.001
Hypercholesterolemia (%)	19.2	22.8	20.7	18.5	19.3	14.6	0.294
drugs (%)	7.6	5.7	5.2	10.9	10.4	5.7	0.757
Current smoking (%)	16.6	23.7	19.7	14.7	15.6	9.4	0.001
Atrial fibrillation (%)	3.0	1.4	0.0	0.0	2.4	11.3	< 0.001
Hemoglobin bA1c (%)	7.2 $\pm$ 1.4	7.5 $\pm$ 1.6	7.3 $\pm$ 1.5	7.2 $\pm$ 1.2	7.1 $\pm$ 1.3	7.0 $\pm$ 1.1	0.001
Anti-DM drugs (%)	45.4	35.5	40.8	49.8	47.2	53.8	0.165
eGFR (mL/minute/1.73m ²)	76.1 $\pm$ 17.5	81.3 $\pm$ 16.7	81.1 $\pm$ 18.9	75.2 $\pm$ 16.6	75.7 $\pm$ 16.2	67.3 $\pm$ 15.3	< 0.001

	UACR					P^*
	Q1 (n = 212)	Q2 (n = 212)	Q3 (n = 212)	Q4 (n = 211)	Q5 (n = 212)	
Inter quartile level	5.2-8.8	11.5-15.4	19.7-26.3	36.0-60.3	116.2-508.9	
Age (years)	63.9 $\pm$ 9.4	64.4 $\pm$ 9.0	66.9 $\pm$ 8.3	68.1 $\pm$ 8.9	67.0 $\pm$ 9.1	< 0.001
Male/Female	117/95	94/118	86/126	97/114	115/97	0.008
BMI (kg/m ²)	24.6 $\pm$ 3.3	24.9 $\pm$ 3.3	24.8 $\pm$ 3.4	25.5 $\pm$ 3.9	25.9 $\pm$ 4.3	0.001
Systolic BP (mmHg)	125 $\pm$ 17	129 $\pm$ 18	130 $\pm$ 16	138 $\pm$ 19	144 $\pm$ 21	< 0.001
Diastolic BP (mmHg)	73 $\pm$ 10	75 $\pm$ 10	76 $\pm$ 10	78 $\pm$ 10	81 $\pm$ 11	< 0.001
Hypertension (%)	46.0	48.6	52.8	67.8	75.9	< 0.001
drugs (%)	29.7	32.5	36.3	46.9	51.4	< 0.001
Hypercholesterolemia (%)	19.3	18.4	17.9	17.5	22.6	0.680
drugs (%)	9.4	5.2	9.0	7.6	6.6	0.642
Current smoking (%)	18.4	15.6	15.6	16.6	17.0	0.933
Atrial fibrillation (%)	2.3	1.9	3.3	2.8	4.7	0.493
Hemoglobin bA1c (%)	7.0 $\pm$ 1.1	7.1 $\pm$ 1.3	7.0 $\pm$ 1.2	7.2 $\pm$ 1.2	7.8 $\pm$ 1.7	< 0.001
Anti-DM drugs (%)	40.6	47.6	44.8	41.2	52.8	0.224
eGFR (mL/minute/1.73m ²)	75.0 $\pm$ 14.1	76.4 $\pm$ 15.4	76.8 $\pm$ 17.0	77.4 $\pm$ 17.3	75.1 $\pm$ 22.6	0.555

BMI indicates body mass index; BP, blood pressure; DM, diabetes; and eGFR, estimated glomerular filtration rate. * Among quintiles.

0.001), but not with UACR ($r = 0.039$, $P = 0.201$).

The cohorts were followed for 6,010 person-years. Composite CV events (heart failure, acute myocardial infarction,

Figure 1. Kaplan-Meier curves of cumulative CV event-free rate according to quintiles of plasma BNP (left) and UACR (right) at baseline in the DM cohort.

Table II. Event Rates and Hazards Ratios for CV Events by Quintiles of Plasma BNP and UACR in DM Cohort

	CV events /1000 py	Sex-age adjusted HR (95%CI)	P	Multi-adjusted HR (95%CI)*	P
BNP					
Q1	3.9	Reference		Reference	
Q2	6.6	1.44 (0.47 - 4.42)	0.552	1.68 (0.54 - 5.18)	0.368
Q3	6.6	1.34 (0.43 - 4.15)	0.612	1.51 (0.49 - 4.70)	0.476
Q4	11.8	2.36 (0.83 - 6.69)	0.106	2.26 (0.80 - 6.41)	0.125
Q5	27.2	4.91 (1.82 - 13.26)	0.002	4.38 (1.69 - 11.84)	0.004
		P for trend < 0.001		P for trend = 0.008	
UACR					
Q1	7.4	Reference		Reference	
Q2	5.7	0.82 (0.31 - 2.21)	0.699	0.80 (0.30 - 2.16)	0.657
Q3	15.2	1.93 (0.86 - 4.32)	0.108	1.89 (0.84 - 4.25)	0.124
Q4	8.4	0.94 (0.38 - 2.33)	0.894	0.77 (0.30 - 2.00)	0.600
Q5	18.0	2.07 (0.94 - 4.54)	0.069	1.47 (0.64 - 3.40)	0.365
		P for trend = 0.05		P for trend = 0.106	

* Adjusted by sex, age, systolic BP, diastolic BP, BMI, HbA1c, CV histories, smoking, hyperlipidemia, Af, eGFR, use of antihypertensive drugs, and use of anti-DM drugs.

stroke) during the follow-up period (mean = 5.7 years) were found in 65 cases in the DM cohort. The number of CV events per 1,000 person-years was 10.8. The Kaplan-Meier curves for CV event-free rate according to quintile levels of BNP and of UACR in the DM cohort are shown in Figure 1. The CV event-free rate was significantly lower in the highest BNP quintile (IQR; 46.5 to 62.3 pg/mL; $P < 0.001$ by log-rank tests). However, for UACR, although the log-rank test showed a significant difference in CV event-free curves among the quintiles ($P = 0.014$), no linear relationships were found between the levels of UACR and CV event-free rate (Figure 1). When age-stratified analysis (divided by median age) was performed in the case of BNP, similar results were obtained (above median; $P = 0.0029$; below median; $P = 0.0021$).

As shown in Table II, the number of CV events per 1,000 person-years increased according to quintile levels for both markers. After adjustment for age and sex, Cox regression analysis was performed to analyze the relationship between plasma BNP levels and risk of CV events among the quintiles. The hazard ratio (HR) for the highest quintile of plasma BNP was significantly higher than that for the lowest quintile (HR = 4.91; 95% CI = 1.82 to 13.26; $P < 0.002$). In addition, in a multivariate adjustment of the model, the relationship between

Figure 2. Multivariable adjusted hazard ratios of the risk of CV events according to quintiles of plasma BNP (black bars) and UACR (gray bars).

Figure 3. The area under the receiver operating characteristic curves of the Framingham CV risk score with and without BNP (left) and UACR (right) in DM cohort. The area under the curve (AUC) was significantly improved when BNP testing was added to the Framingham CV risk score.

plasma BNP quintiles and CV event rate remained robust (HR = 4.38; 95%CI = 1.69 to 11.84; $P < 0.004$; Table II). As shown in Figure 2, there was a linear association between BNP levels and HR for CV events (P for trend = 0.008). However, in the case of UACR, no significant association was found between quintile and CV events.

The AUC of the ROC analysis for CV prediction was 0.66 (95%CI, 0.63 to 0.69) for FRS and 0.69 (95%CI, 0.66 to 0.72) for BNP (not significant). As shown in Figure 3, when BNP was added to FRS, the ROC analysis AUC (0.74; 95%CI, 0.71 to 0.76) was significantly increased compared to that for FRS alone ($P = 0.05$). In contrast, when UACR (AUC = 0.61; 95%CI, 0.58 to 0.64) was added to FRS the overall predictive ability in terms of AUC generated from ROC analysis did not change (AUC = 0.67; 95%CI, 0.66 – 0.71; $P = 0.49$).

DISCUSSION

The present study has demonstrated that elevated plasma BNP at 5th quintile levels are useful for CV risk stratification in the DM population independent of glycemic control and accumulation of established CV risk factors. Moreover, the present findings suggest that the addition of BNP testing to the FRS improves its value for CV risk prediction.

Tarnow, *et al* reported that, in 315 patients with type-2 DM, the highest NT-proBNP tertile level had a 2.7 times higher risk for all-cause mortality than the lowest tertile in a long-term follow-up study (19 years).⁸⁾ Huelsmann, *et al* have shown that DM subjects with NT-proBNP levels less than 125 pg/mL had an excellent short-term prognosis for unplanned hospitalization for CV events and death.⁵⁾ These previous single center studies were performed under specialist diabetes care and did not identify the threshold level of plasma BNP indicating increased risk of CV events. In a general practical context where health care providers are usually caring for large numbers of patients with DM, it would be helpful to be able to identify individuals at high risk of CV events by a reliable and simple blood test.

The present study has shown for the first time that a plasma BNP level of approximately 50 pg/mL is a signal of increased CV risk in a real world DM population. This value may be a useful marker for the implementation of intensive intervention with multiple drug combinations and behavior modification in these patients. The association between elevated plasma BNP and CV events may be due to the fact that increased plasma BNP is a biomarker for asymptomatic structural heart disease such as impaired left ventricular function, left ventricular hypertrophy, atrial dilatation and fibrillation, valvular heart disease, and myocardial ischemia.^{20,21)} In our previous cross-sectional study applying transthoracic echocardiography in the general population, plasma BNP concentrations over 50 pg/mL showed sensitivity and specificity for several select phenotypes of structural heart disease which are prone to progress into several types of CV events.²²⁾

Although obese subjects with insulin resistance as represented by type-2 DM are expected to have relatively low plasma BNP levels,²³⁾ the threshold in this group is higher than in the low risk general population as previously reported.³⁾ The differences in these levels may be due to the relatively high prevalence of left ventricular diastolic dysfunction, hypertro-

phy, coronary heart disease, renal dysfunction, and anemia in DM subjects. All of these cardiac and noncardiac factors increase plasma BNP levels, and are obvious substrates to facilitate CV events.

Urine albumin excretion is suggestive of glomerular permeability, with increasing levels of albumin in the urine indicating extensive glomerular endothelial damage and serving as a marker of systemic microvasculopathy. Several studies have shown that abnormally elevated urinary albumin excretion, microalbuminuria, is predictive of risk for all-cause and CV mortality in DM subjects.^{24,25)} However, levels of urinary albumin excretion may not be directly related to severity of CV functional and structural abnormality. In the present results, levels of UACR were not directly correlated with CV event risk in either univariate or multivariate analysis. In contrast, the association between BNP levels and CV events followed a linear dose-effect relationship over time. These findings suggest that plasma BNP levels are a biomarker for a wide range of clinical or subclinical pancardiac target organ damage,²⁶⁾ and thus may be superior to UACR for CV risk stratification in subjects with DM. However, no studies have examined the effect of adding these two biomarkers to an established CV risk prediction model in terms of ability to discriminate between cases and noncases. The present study has demonstrated that the C-statistics of the ROC analysis for CV prediction by BNP testing were comparable to the FRS, and improved significantly when BNP testing was added to the established CV risk model. In view of these findings, plasma BNP measurement appears to be more useful than UACR as a marker of total CV risk in subjects with DM.

The present study had several strengths. The study cohort included a large number of subjects with DM (> 1,000). This group may be representative of the real world situation because these subjects were recruited from a community-based population irrespective of anti-DM medication status and glycemic control levels. Plasma BNP measurement was performed in fresh plasma samples without long-term freezing and repeated thawing. CV events were captured prospectively according to previously determined standard epidemiological criteria and confirmed by research staff at medical chart review. Several important biochemical parameters such as UACR and HbA1c at baseline were determined concomitantly before the start of the follow-up study.

Despite the merits delineated above, several limitations must be considered when interpreting the results. First, DM subjects were defined mainly by a single HbA1c measurement. However, Selvin, *et al* reported that the within-person coefficient of variation in repeated HbA1c measurements was less than 4% in a community-based study.²⁷⁾ Second, mean BMI was lower in our study than in previous DM populations.^{5,8)} Cardiac natriuretic peptides in plasma have been reported to be lower in subjects with obesity and insulin resistance than in nonobese and insulin sensitive subjects.²³⁾ Consequently, threshold BNP levels may be lower in a predominantly obese DM population. Third, the outcome of this study did not include all types of coronary artery disease such as angina pectoris and sudden cardiac death. Further studies are needed to validate the present results in other ethnic populations with a higher prevalence of coronary artery disease. Lastly, the effects of adding BNP to the FRS in terms of ability to discriminate between cases with and without CV events were evaluated by

a less sensitive method such as c-statistics (AUC of ROC analysis) rather than the recently developed event-specific reclassification tables and integrated discrimination improvement.²⁸⁾ However, the present study showed a significant improvement in the AUC. This means that, even using the traditional statistical method, the discriminatory abilities were clearly enhanced by the addition of BNP to the established CV risk model in subjects with DM.

In conclusion, in a community-based sample of people with DM, plasma BNP levels above the 80th percentile are directly associated with CV risk. Measurement of plasma BNP alone or with an established CV risk score is of value for predicting CV events in these subjects.

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