

# Correlation between brain natriuretic peptide levels and the prognosis of patients with left ventricular diastolic dysfunction

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**Abstract.** The present study aimed to investigate the association between brain natriuretic peptide (BNP) levels and the prognosis of patients with left ventricular (LV) diastolic dysfunction. A total of 708 inpatients with cardiovascular disease (mean age, 66 years; 395 males and 313 females) were grouped according to initial BNP and were followed-up for 20-51 months (average, 30.86 months) until endpoint events occurred. Endpoints were defined as mortality or readmission due to cardiovascular disease, or mortality due to any other reason. A total of 67 and 77 events were reported in the BNP  $\leq 80$  pg/ml and BNP  $> 80$  pg/ml groups, respectively. The occurrence rate of the endpoint was significantly higher in the BNP  $> 80$  pg/ml group, as compared with the BNP  $\leq 80$  pg/ml group (26.28 vs. 16.14%; relative risk=1.63). Furthermore, the durations of patient survival were significantly shorter in the BNP  $> 80$  pg/ml group, as compared with the BNP  $\leq 80$  pg/ml group ( $P=0.0006$ ), and patient survival decreased as BNP levels rose ( $P=0.0074$ ). Among the 708 patients, 677 underwent echocardiographic detection at the same time. No significant correlation was detected between BNP levels and survival time in 178 patients with normal LV diastolic function [mitral Doppler flow, early diastolic (E)/late diastolic (A) $>1$ ] ( $P=0.2165$ ); whereas a negative correlation was determined in 499 patients with LVD dysfunction ( $E/A \leq 1$ ) (Spearman's  $\rho = -0.0899$ ;  $P=0.0447$ ). The prognoses of patients with elevated BNP levels were correspondingly worse in the present study and these correlations were demonstrated to be significant in patients with LV diastolic dysfunction. Therefore, BNP levels may be used to predict the prognosis of patients with cardiovascular disease.

## Introduction

Brain natriuretic peptide (BNP) is a 32-amino acid peptide which functions as a cardiac neurohormone that is predominantly released from the cardiac ventricle in response to left ventricular (LV) volume expansion and pressure overload (1). As such, BNP is a potent vasodilator and a natriuretic factor that regulates salt and water homeostasis. Increased secretion of BNP predominantly occurs in response to increased tension in the ventricular walls, decreased oxygen supply, acute myocardial infarction (MI), chronic cardiac heart failure (HF) and during hypertrophy of the heart (2). It has previously been demonstrated that BNP levels are useful for the diagnosis of LV systolic and diastolic dysfunction, and these levels correlate with the severity and prognosis of the dysfunction (3). As BNP is predominantly released from the LV myocardium, it is necessary to measure the regional function of the sympathetic nervous system in the left ventricle in order to elucidate the mechanisms associated with the release of BNP release and the clinical significance of the increased BNP levels during HF (4).

The prognostic importance of BNP and the N-terminal of pro (NT-pro)BNP has previously been investigated in patients with acute coronary syndromes and patients with HF, and both markers were shown to be strong predictors of mortality and morbidity (5). In particular, diastolic wall stress has been demonstrated to have a stronger correlation with NT-proBNP levels, as compared with systolic wall stress (6). Previous studies have suggested that the estimation of BNP values via a fast and reliable blood test may be accepted as a diagnostic tool for assessing asymptomatic diastolic dysfunction in patients with hypertrophic cardiomyopathy (HCM), diabetes and hypertension (7-9). Furthermore, as BNP has been demonstrated to be a highly useful, simple and noninvasive diagnostic biomarker, BNP levels may be used for the differential diagnosis of cardiac and pulmonary dyspnea in emergency departments to guide therapy (10). However, whether BNP levels are predictors of morbidity and mortality in patients with diastolic dysfunction is yet to be investigated. Therefore, the present observational study aimed to elucidate the effects of BNP levels on the survival time of patients with diastolic dysfunction by grouping and following up 708 inpatients with cardiovascular disease until endpoint events occurred.

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## Materials and methods

**Ethics statement.** The data collection in the present study was conducted according to the principles of the Declaration of Helsinki ([https://en.wikipedia.org/wiki/Declaration\\_of\\_Helsinki](https://en.wikipedia.org/wiki/Declaration_of_Helsinki)), and this process did not affect the normal course of therapy. All data were analyzed anonymously. The protocol of the present study was approved by the Ethics committee of the hospital, and written informed consent was obtained from each patient.

**Subjects and procedures.** In the present observational study, 708 inpatients with cardiovascular disease at the Division of Cardiology (Jinshan Hospital of Fudan University) were followed-up between May 2006 and December 2008 until endpoints occurred. The duration of follow-up ranged between 20 and 51 months and averaged 30.86 months. The mean age of patients was 66 years, and the majority of the patients were male (395/708; 55.8%). Patients were classified according to an initial BNP cut-off point of 80 pg/ml. Endpoints were defined as mortality or readmission due to cardiovascular disease, or mortality due to any other reason.

**Measurement of plasma BNP concentration.** Blood samples for the analysis of plasma BNP levels were harvested from the antecubital vein upon patient admission. BNP levels were analyzed using the Triage® BNP Test (Biosite Diagnostics, Inc., San Diego, CA, USA), which is a single-use fluorescence immunoassay device that is designed to determine BNP levels in ethylenediaminetetraacetic acid-anticoagulated whole blood or plasma specimens (EDTA purchased from Biosite Diagnostics, Inc.). Briefly, the specimen was added to the sample port of the test device using a 250- $\mu$ l transfer pipette and the device was inserted into the Triage® meter (Biosite Diagnostics, Inc.). BNP analysis was automatically conducted after the sample had reacted with the reagents within the BNP device. Reaction and analysis lasted ~15 min. BNP quantification was conducted based on the quantity of fluorescence detected by the meter within the measurement zone on the device, where increased fluorescence indicated a higher concentration of BNP in the specimen (11).

**Echocardiography.** M-mode and two-dimensional images, and color flow Doppler recordings were obtained using Vivid-7 ultrasound system (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) operating at 2.0-3.5 MHz, as previously described (11). LV ejection fraction (LVEF) was calculated according to the Simpson formula outlined by Teichholz *et al* (12). In the present study, LVEF  $\geq$ 50% indicated that LV systolic function was normal, whereas LVEF <50% indicated systolic dysfunction. A 7.5 MHz pulsed Doppler transducer (GE Healthcare Bio-Sciences) was used to measure diastolic transmitral Doppler parameters, including the peaks of early (E) and late diastolic (A) mitral flow velocities. Diastolic dysfunction was defined as an E/A ratio  $\leq$ 1.

**Statistical analysis.** Baseline characteristics of patients enrolled in the present were presented as percentages and medians with interquartile ranges for dichotomous and continuous variables, respectively. Between-group baseline characteristics

comparisons were performed using Wilcoxon rank-sum test and the  $\chi^2$  test for continuous and variables discrete, respectively. Survival curves were generated using Kaplan-Meier estimates; between-group differences in survival duration were compared using the log-rank test. Between-groups differences in the incidence of endpoint events were evaluated via relative risk (RR).

Spearman correlation was used to correlate continuous data with BNP levels and the duration of patients survival. BNP levels were evaluated via receiver operating characteristic (ROC) and area under curve (AUC) analyses for the prediction of clinical endpoints. In order to determine optimal specificity (Sp) and sensitivity (Se) values the value closest to the peak Sp and Se points on the ROC curve was identified.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Clinical characteristics.** The baseline clinical characteristics of the patient population investigated in the present study are shown in Table I. Patient risk factors included: Hypertension (n=390; 55%); diabetes (n=147; 21%); hyperlipidemia (n=101; 14%); renal dysfunction, which was defined by serum creatinine  $>84$  or  $>104$   $\mu$ mol/l in females and males, respectively (n=115; 16%); MI (n=90; 13%); and pharmacological intervention, including treatment with  $\beta$ -blockers, calcium antagonists, diuretics, nitrates, antiplatelet agents, statins, angiotensin converting enzyme inhibitor and angiotensin receptor blocker. A total of 415 and 293 patients exhibited BNP  $\leq$ 80 and  $>80$  pg/ml, respectively. During follow-up, 144 endpoint events occurred, including 67 and 77 in the BNP  $\leq$ 80 pg/ml and  $>80$  pg/ml groups, respectively. Among the two patient groups, mortality and readmission rates were 32 (4.5%) and 112 (15.8%), respectively. Mortality rates were significantly higher in the BNP  $>80$  pg/ml group, as compared with the BNP  $\leq$ 80 pg/ml group ( $P < 0.05$ ); whereas readmission was markedly elevated in the BNP  $\leq$ 80 pg/ml group, as compared with the BNP  $>80$  pg/ml group. A total of 677 patients underwent echocardiograph detection at the same time, including 499 (73.7%) patients with diastolic dysfunction and 277 (40.9%) patients with systolic dysfunction. The incidence of endpoint events was significantly elevated in the BNP  $>80$  pg/ml, as compared with the BNP  $\leq$ 80 pg/ml group (26.28 vs. 16.14%; RR=1.63).

**Patient survival times decrease as BNP levels increase.** Kaplan-Meier analysis survival curves of patients grouped according to initial BNP levels are shown in Fig. 1. This analysis was performed in order to investigate whether the survival time of patients in the BNP  $\leq$ 80 pg/ml group was increased compared with patients in the BNP  $>80$  pg/ml group. The results demonstrated that patients in the BNP  $\leq$ 80 pg/ml group survived significantly longer, as compared with the BNP  $>80$  pg/ml group ( $P = 0.0006$ ; log rank=11.74).

Spearman correlation analysis demonstrated that the survival time of the 708 patients enrolled in the present study markedly decreased as BNP levels increased (Spearman's rho=-0.1006;  $P = 0.0074$ ). This negative correlation between the log BNP levels and survival time is shown in Fig. 2. No significant correlation was detected between BNP

Table I. Baseline clinical characteristics of patients grouped according to BNP level.

Parameter	BNP $\leq$ 80 pg/ml (%)	BNP >80 pg/ml (%)	P-value
Patients (n)	415	293	-
Age (years)	62 (54-72)	74 (64-78.75)	<0.05
Male (%)	226 (54)	169 (57)	0.395
Mortality (%)	3 (0.72)	29 (9.90)	<0.05
Readmission (%)	64 (15.42)	48 (16.38)	0.73
Endpoint events	67 (16.14)	77 (26.28)	0.001
Diastolic dysfunction	297/397 (74.81)	202/280 (72.14)	0.451
Systolic dysfunction	112/397 (28.21)	165/280 (58.93)	<0.05
Hypertension	208 (50.12)	182 (62.12)	0.002
Diabetes	77 (18.55)	70 (23.89)	0.085
Hyperlipidemia	74 (17.83)	27 (9.22)	0.001
Renal failure	29 (6.99)	86 (29.35)	<0.05
Myocardial infarction	43 (10.36)	47 (16.04)	0.025
Medications:			
$\beta$ -blockers	245 (59.04)	148 (50.51)	0.025
Calcium antagonists	139 (33.49)	99 (33.79)	0.935
Diuretic	126 (30.36)	230 (78.50)	<0.05
Nitrates	214 (51.57)	141 (48.12)	0.367
Antiplatelet agents	328 (79.04)	217 (74.06)	0.121
Statins	118 (28.43)	79 (26.96)	0.667
ACEI or ARB	323 (77.83)	239 (81.57)	0.226
ACEI	168 (40.48)	127 (43.34)	0.447
ARB	155 (37.35)	112 (38.23)	0.813

BNP, brain natriuretic peptide; ACE, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker. Wilcoxon rank-sum test and the  $\chi^2$  test was used.

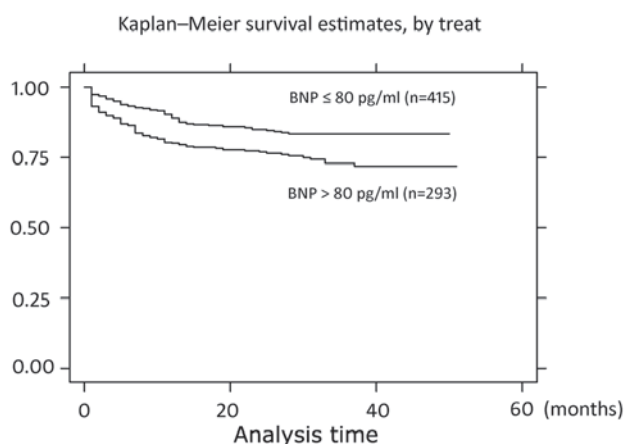


Figure 1. Kaplan-Meier survival analysis of the patients with plasma BNP levels higher and lower than the 80 pg/ml cut-off value.  $P=0.0006$ ; log rank=11.74. BNP, brain natriuretic peptide.

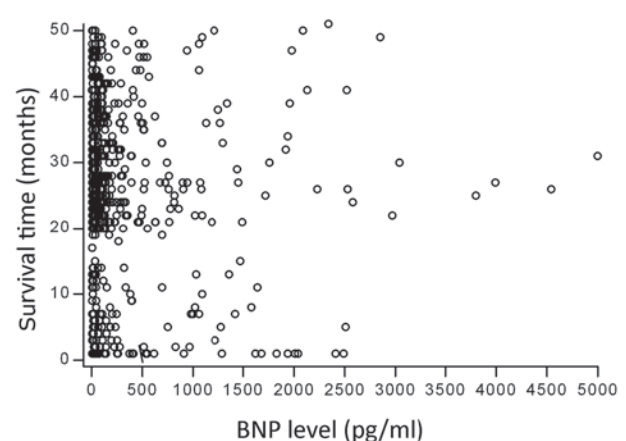


Figure 2. Scatter plots demonstrating the correlation between log BNP levels and survival time in all patients ( $n=708$ ). BNP, brain natriuretic peptide.

levels and survival time in the 178 patients with normal LV diastolic function (Spearman's  $\rho=-0.931$ ;  $P=0.2165$ ) and 277 patients with systolic dysfunction (Spearman's  $\rho=-0.0007$ ;  $P=0.9904$ ) whereas a negative correlation was detected in the 499 patients with LV diastolic dysfunction ( $E/A \leq 1$ ) (Spearman's  $\rho=-0.0899$ ;  $P=0.0447$ ). A scatter plot

demonstrating the correlation between the log BNP level and duration of survival in patients with diastolic dysfunction are shown in Fig. 3.

*BNP levels can predict clinical endpoints in patients with diastolic dysfunction.* The predictive utility of plasma BNP

Table II. Correlation between the log BNP level and endpoint events in all patients.

BNP level	Endpoint events		Total
	Yes	No	
>80 pg/ml	77	216	293
≤80 pg/ml	67	348	415
Total	144	564	708

The  $\chi^2$  test was used. BNP, brain natriuretic peptide.

Table III. Correlation between the log BNP level and endpoint events in patients with diastolic dysfunction.

BNP level	Endpoint events		Total
	Yes	No	
>80 pg/ml	48	154	202
≤80 pg/ml	44	253	297
Total	92	407	499

The  $\chi^2$  test was used. BNP, brain natriuretic peptide.

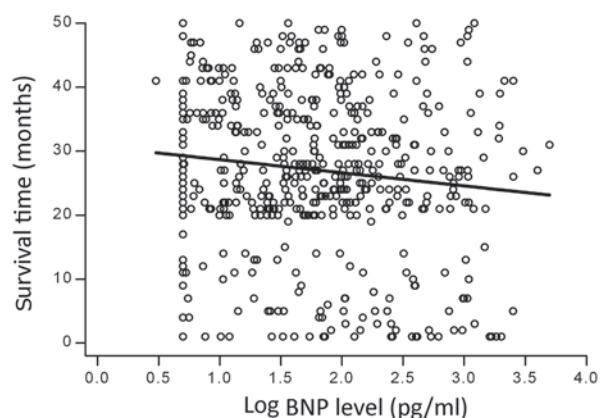


Figure 3. Scatter plots demonstrating the correlation between log BNP levels and survival time in patients with diastolic dysfunction (n=499). BNP, brain natriuretic peptide.

levels for the determination of compound clinical endpoints was calculated via ROC analysis in all patients. The area under the ROC curve was 0.6127 [standard error (SE)=0.0268; 95% confidence interval (CI) 0.56015-0.66531]. The cut-off value of the plasma BNP level was 80 pg/ml (Se, 53.47%; Sp, 61.7%) (Fig. 4 and Table II). In patients with diastolic dysfunction, the area under the ROC curve was 0.5977 (SE=0.0345; 95% CI, 0.53020-0.66524). In this group, the cut-off value of the plasma BNP level was 80 pg/ml (Se, 52.17%; Sp, 62.16%) (Fig. 5 and Table III). No significant correlation was detected between plasma BNP levels and the incidence of endpoint events in patients with systolic dysfunction.

## Discussion

BNP is a cardiac neurohormone secreted from the ventricles into the plasma in response to ventricular volume expansion and pressure overload (3). It has previously been demonstrated that the determination of BNP levels provides a straightforward method for the early detection of HF, the assessment of HF severity and the effectiveness of treatment (13). A previous study (14) has shown that both BNP and NT-proBNP are strong prognostic markers for numerous acute coronary syndromes, including patients with unstable angina, non-ST and ST-elevation MI (15-17) and stable angina pectoris (18,19). In the absence of significant necrosis (20), BNP and NT-proBNP are present in human coronary

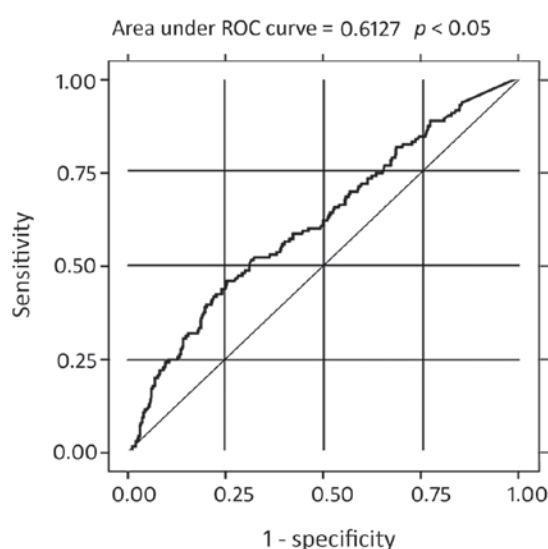


Figure 4. ROC analysis of log brain natriuretic peptide levels for determining compound clinical endpoint events in all patients (n=708). Area under the curve=0.6127 and standard error=0.0268 (95% confidence interval, 0.56015-0.66531). The cut-off value was determined to be 80 pg/ml (specificity, 61.7%; sensitivity, 53.47%). ROC, receiver operating characteristic.

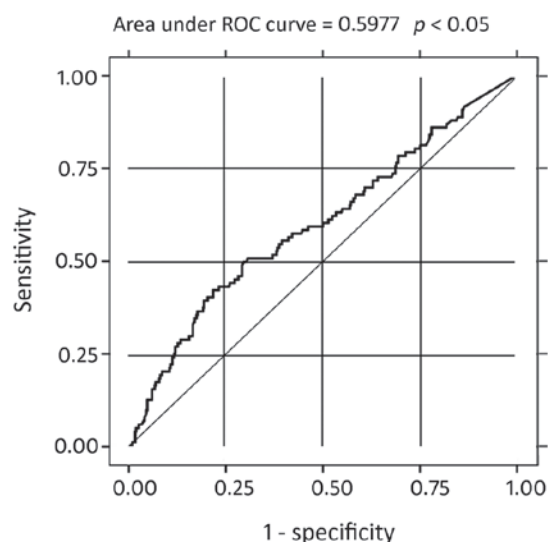


Figure 5. ROC analysis of log brain natriuretic peptide levels for determining compound clinical endpoint events in patients with diastolic dysfunction (n=499). Area under the curve=0.5977 and standard error=0.0345 (95% confidence interval, 0.53020-0.66524). The cut-off value was determined to be 80 pg/ml (specificity, 62.16%; sensitivity, 52.17%). ROC, receiver operating characteristic.



arteries (21) and are associated with the extent and severity of coronary atherosclerotic lesions (22). The results of the present study were consistent with these previous findings; the number of patients who suffered a MI was significantly elevated in the BNP >80 pg/ml group, as compared with the BNP ≤80 pg/ml group ( $P=0.025$ ). It has previously been suggested that ischemia may act as a stimulus for the release of BNP and NT-proBNP (23). Sympathetic nervous overactivity in the left ventricle has been demonstrated to be an important mechanism for the induction of BNP elevation in chronic ischemic HF (4). Furthermore, previous studies have shown that ventricular BNP gene expression is upregulated by acute myocardial hypoxia, which results in increased plasma BNP and proBNP levels (24,25).

NT-proBNP is independent of invasive measurements of LV function and the severity of coronary artery disease (19). Previous studies have investigated the prognostic importance of measuring BNP and NT-proBNP levels in patients with HF and acute coronary syndromes, and both markers were demonstrated to be strong and independent predictors of morbidity and mortality (19,26,27). These predictors were also detected in >60% of the subgroup of patients with LV ejection fraction and in patients with diabetes mellitus (28). Kragelund *et al* (19) demonstrated that, when measured immediately prior to coronary angiography, NT-proBNP provides prognostic information on mortality in patients with stable coronary heart disease (19). The results of the present study were consistent with these findings and suggest that BNP demonstrates the same prognostic value as previous studies. The incidence of clinical endpoints, which included mortality or readmission due to cardiovascular disease, or mortality due to any other reason, was significantly higher in the BNP >80 pg/ml group, as compared with the BNP ≤80 pg/ml group. Furthermore, Kaplan-Meier analysis survival curves suggested a negative correlation between the log BNP level and survival duration in patients with cardiovascular disease. Patients in the BNP ≤80 pg/ml group survived significantly longer, as compared with the BNP >80 pg/ml group ( $P=0.0006$ , log rank=11.74); therefore, survival duration reduced as BNP levels increased.

In the present study, a cut-off value of 80 pg/ml plasma BNP was used for the prediction of cardiovascular morbidity and mortality from all death causes, including cardiovascular diseases (Se, 53.47%; Sp, 61.7%). BNP or NT-proBNP have previously been shown to have a high negative predictive value as a single test (29), and the results of the present study were consistent with these findings. The present study assessed inpatients with various diseases, including, but not limited to, hypertension, diabetes, hyperlipidemia, renal failure and MI, between May 2006 and December 2008 at the Division of Cardiology (Jinshan Hospital of Fudan University). The results demonstrated that BNP levels may be used for the prediction of various cardiovascular diseases, rather than one or several specific diseases. Therefore, the present study demonstrated the wider application of BNP as a predictor, as compared with previous studies (7-9).

A previous study has demonstrated that 40-50% of individuals with HF have a normal ejection fraction, and diastolic dysfunction is the presumed cause of diastolic HF (DHF) (30). Abnormalities in diastolic function are often misdiagnosed as

this condition does not always produce signs and symptoms of HF, which leaves patients predisposed to symptomatic HF and cardiac necrosis due to the delay in treatment (31). The early diagnosis of LV diastolic dysfunction during its initial phase facilitates the initiation of effective treatment with the purpose of halting the progress of the disease and delaying the development of symptomatic HF (32). Echocardiography, using both Doppler measurements of transmitral and pulmonary vein blood flow velocities and tissue Doppler imaging, is a widely accepted method for the clinical analysis of diastole (33). However, these assessments are: i) Costly due to the requirement of complex equipment; ii) time-consuming, as they involves the analysis of various variables; and iii) difficult, therefore they must be performed by a trained operator (34). Therefore, a simple, objective, high sensitivity and high specificity method for the evaluation of diastole function is required. An association between BNP levels and the indices of diastolic function has previously been described in patients with reduced and preserved LVEF (35). Previous studies have demonstrated that the estimation of BNP values provides a fast and reliable blood test in the diagnosis of asymptomatic diastolic dysfunction (7,8). In particular, the BNP test has previously been evaluated for the prediction of asymptomatic diastolic dysfunction in patients with hypertension (7); and BNP levels may be determined in order to evaluate occult LV dysfunction in patients who are periodically assessed for diabetic complications (8). Furthermore, Panou *et al* (9) have demonstrated that BNP assessment may be used as an adjunctive, reliable and objective way of estimating cardiac dysfunction in HCM (9). These findings were supported by a subsequent study that demonstrated that assessment of BNP levels can be easily and rapidly performed in emergency departments to guide therapy, follow the response to therapy and predict the exercise capacity of patients (10). The results of the present study indicated that patients with elevated BNP levels suffered poorer prognoses, as compared with those with lower BNP levels. A negative correlation between BNP levels and survival time was detected in patients with LV diastolic dysfunction ( $n=499$ ), and survival time decreased as BNP levels rose. Furthermore, the predictive utility of plasma BNP levels for the determination of compound clinical endpoints was demonstrated in patients with diastolic dysfunction. In patients with diastolic dysfunction enrolled in the present study, morbidity and mortality associated with cardiovascular diseases were increased in patients with elevated plasma BNP levels, as compared with patients with lower BNP levels. In patients with systolic dysfunction, no significant correlation or predictive value was detected between plasma BNP levels and survival time and morbidity and mortality from cardiovascular diseases.

The findings of the present study are consistent with a previous study which has suggested that diastolic wall stress may have a stronger correlation with NT-proBNP levels, as compared with systolic wall stress (6). In a previous study, BNP levels exhibited higher sensitivity (85 vs. 63%) and positive predictive values (69 vs. 55%), as compared with the assessment of NT-proBNP levels; although the negative predictive values of BNP and NT-proBNP remained similar (70 and 71%, respectively) (36). Furthermore, measuring BNP levels has been demonstrated to have a higher sensitivity and

positive predictive value for the accurate diagnosis of severe LVSD, as compared with measuring NT-proBNP levels (36). The plasma half-life of BNP in humans is ~20 min, whereas the circulating half-life of NT-proBNP is ~120 min (37). Therefore, BNP level determination may facilitate assessment of the current severity of LV dysfunction, guide therapy and track the immediate response to therapy, whereas the use of NT-proBNP levels for assessment would lag by ~10 h. Clearance of BNP occurs via two main mechanisms: i) Binding to clearance receptors; and ii) enzymatic degradation by neutral endopeptidase (38). Clearance of NT-proBNP predominantly occurs via the kidney and NT-proBNP levels significantly rise during mild renal failure, which seriously affects the utility of NT-proBNP for diagnosis (39,40), as ~29% of patients with HF suffer from renal failure (41). In patients aged  $\geq 65$ , the BNP assay is a more useful diagnostic indicator for cardiogenic pulmonary edema, as compared with proBNP (39). The estimated glomerular filtration rate has independent effects upon plasma BNP and NT-proBNP concentrations in patients with chronic kidney disease, and NT-proBNP appears to be more severely affected by declining kidney function (42). Therefore, based on these findings and the results of the present study, the use of plasma BNP levels may result in a reliable and effective assessment of prognosis in patients with cardiovascular diseases.

The present study had several limitations. Firstly, the number of patients enrolled in the study was small and the follow-up period was relatively short. Therefore, future studies with a larger sample of patients should be conducted over a longer period in order to assess the predictive value of BNP in patients with cardiovascular-related disease, particularly patients with LV diastolic dysfunction. Secondly, echocardiographic parameters should be interpreted with caution as the E/A may be affected by rapid ventricular rate and atrial fibrillation. Thirdly, the sensitivity and specificity of the predictive utility of plasma BNP levels for the determination of compound clinical endpoints in all patients or patients with LV diastolic dysfunction was not particularly high. In a previous study, the combination of NT-proBNP or BNP with LVEF substantially improved the risk stratification for mortality, HF, and new ischemic events (43).

In conclusion, the prognoses of patients with elevated BNP levels were correspondingly worse when compared with patients with lower BNP levels. This correlation was demonstrated to be significant in patients with LV diastolic dysfunction. Therefore, the results of the present study suggested that BNP levels may be used to predict the prognosis of patients with cardiovascular disease, particularly in patients with LV diastolic dysfunction.

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