Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.0471-17
Intern Med 57: 2621-2630, 2018
http://internmed.jp

【 ORIGINAL ARTICLE 】

The Molar Ratio of N-terminal pro-B-type Natriuretic Peptide/B-type Natriuretic Peptide for Heart Failure-related Events in Stable Outpatients with Cardiovascular Risk Factors

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Abstract:

Objective B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) should be secreted from cardiomyocytes in response to increased myocardial wall stress in a molar ratio of 1.00; however, the calculated molar blood levels of NT-proBNP are often greater than those of BNP in routine clinical practice. The purpose of this study was to investigate the hypothesis that the molar ratio of NT-proBNP/BNP provides useful clinical information in stable outpatients with cardiovascular risk factors.

Methods We measured both the BNP and NT-proBNP levels simultaneously in 551 consecutive, stable outpatients with at least one cardiovascular risk factor and then calculated the molar ratio of NT-proBNP/BNP. All patients were prospectively followed-up for the occurrence of heart failure (HF)-related events.

Results Of those patients, 38 patients had an HF-related event. A multivariate Cox hazards analysis showed that the log (molar ratio of NT-proBNP/BNP) was an independent predictor of future HF-related events ($p=0.039$). A Kaplan-Meier analysis showed a significantly higher probability of HF-related events in patients with a higher molar ratio of NT-proBNP/BNP (≥1.70) ($p<0.001$). The area under the curve (AUC) of the receiver operating characteristic curve (ROC) for the molar ratio of NT-proBNP/BNP to predict HF-related events was 0.75 ($p<0.001$). The AUC of the ROC curve analysis with the molar ratio of NT-proBNP/BNP for the prediction of HF-related events was not significantly greater than that of BNP or NT-proBNP.

Conclusion The molar ratio of NT-proBNP/BNP may be a significant prognostic factor for HF-related events.

Key words: molar ratio, B-type natriuretic peptide (BNP), N-terminal pro-B-type natriuretic peptide (NT-proBNP), heart failure, prognosis

(Intern Med 57: 2621-2630, 2018)
(DOI: 10.2169/internalmedicine.0471-17)

Introduction

B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are useful clinical biomarkers for the diagnosis or prognosis of heart failure (HF) (1-8). In response to increased myocardial wall stress due to volume- or pressure-overload, the BNP gene is up-regulated in cardiomyocytes. This results in the production of an intracellular precursor propeptide (proBNP); further processing of this propeptide results in the release of the biologically active BNP and the biologically inert NT-proBNP. Theoretically, BNP and NT-proBNP should be secreted from cardiomyocytes in response to increased myocardial wall stress in a molar ratio of 1.00; however, in routine clinical practice, the calculated molar level of NT-proBNP is often greater than that of BNP in peripheral blood (9-11). BNP is cleared from plasma by binding to the natriuretic peptide receptor type C (NPR-C) and through proteolysis by neutral endopeptidases (NEP; also called

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Received: November 4, 2017; Accepted: February 5, 2018; Advance Publication by J-STAGE: April 27, 2018
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neprilysin). In contrast, NT-proBNP does not bind to NPR-type A (NPR-A) or NPR-C and is not cleaved by NEP. NT-proBNP is cleared from the circulation more slowly than BNP (half-life of 120 vs. 20 minutes) (9, 12). The influence of the renal function on the NT-proBNP clearance seems to be greater than on the BNP clearance (13). As such, the molar ratio of NT-proBNP/BNP might be affected by patients' systemic conditions.

Several studies have reported that conventional cardiovascular risk factors, such as, gender, obesity, hypertension (HT), diabetes mellitus (DM), dyslipidemia (DLP), renal failure, and anemia, are significantly associated with BNP or NT-proBNP levels (1). However, the association between these risk factors and the calculated molar ratio of NT-proBNP/BNP as well as the clinical significance of the molar ratio of NT-proBNP/BNP are still unknown.

In the present prospective study, we examined the baseline clinical factors associated with the molar ratio of NT-proBNP/BNP and whether or not the molar ratio of NT-proBNP/BNP could predict future HF-related events in stable outpatients with cardiovascular risk factors.

Materials and Methods

Study patients

This study recruited 551 consecutive, stable outpatients (264 men, 287 women) who visited Ozawa Clinic with at least 1 cardiovascular risk factor between September 2010 and July 2013. Both BNP and NT-proBNP levels in peripheral blood were measured simultaneously. The cardiovascular risk factors were defined as HT (blood pressure ≥140/90 mmHg or taking antihypertensive medications), DLP [low-density lipoprotein cholesterol (LDL-C) ≥140 mg/dL (3.6 mmol/L), high-density lipoprotein cholesterol (HDL-C) <40 mg/dL (1.04 mmol/L), triglycerides (TG) ≥150 mg/dL (1.7 mmol/L) or taking lipid-lowering medications), DM (fasting blood glucose levels ≥126 mg/dL (7.0 mmol/L), >200 mg/dL (11.1 mmol/L) in an oral glucose tolerance test, or taking anti-diabetic medications], a body mass index (BMI) ≥25 kg/m^2, an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2, and a history of cardiac diseases [a history of coronary artery disease (CAD), left ventricular hypertrophy (LVH) or cardiomyopathy, valve disease (moderate to severe heart valve disease or heart valve replacement), or atrial fibrillation (AF)].

We excluded the patients with acute phase of HF decompensation and acute coronary syndrome who required emergency coronary angiography, defined as either acute myocardial infarction or class II/III unstable angina by Braunwald's classification, at the time of measuring the BNP and NT-proBNP levels. We also excluded the patients with advanced chronic obstructive pulmonary disease, advanced collagen disease, active inflammatory disease, severe liver dysfunction, and neoplasms. Furthermore, we excluded the patients with an eGFR <15 mL/min/1.73 m^2, including those on hemodialysis.

We performed electrocardiogram (ECG) in all recruited 551 patients within 2 months after measuring the BNP and NT-proBNP levels. Of the 551 patients, we performed echocardiography in 462 within 6 months after measuring the BNP and NT-proBNP levels.

All patients were prospectively followed-up for the occurrence of HF-related events every month after measuring the BNP and NT-proBNP levels.

This study was conducted in accordance with the principles contained in the Declaration of Helsinki. The study protocol was approved by the Human Ethics Review Committee of Ozawa Clinic. Signed consent was obtained from each participant. This study was supported by research funds from Ozawa Clinic.

Measurement of the BNP and NT-proBNP levels

The plasma BNP concentration was measured with a specific immunoradiometric assay for human BNP (Shionoria BNP; Shionogi, Osaka, Japan). The serum NT-proBNP concentration was measured with a specific chemiluminescent immunoassay kit (Roche Diagnostics, Basel, Switzerland). To calculate the molar ratio of NT-proBNP/BNP, we transformed the unit of BNP and NT-proBNP from pg/mL to pmol/L using the molecular weights of BNP and NT-proBNP (BNP: 3,464, NT-proBNP: 8,460).

Echocardiography

We used commercially available ultrasound systems (EUB-7500; Hitachi, Tokyo, Japan) to evaluate the cardiac function. Measurement of the LVEF was performed in the biplane apical (2- and 4-chamber) views using a modified Simpson's method. The LV mass was calculated, as described previously (14), and the LV mass index (LVMI) was expressed relative to the body surface area. LV hypertrophy (LVH) was defined as an LVMI >115 g/m^2 (men) or >95 g/m^2 (women).

Follow-up and HF-related events

We followed-up all patients every month with information about HF-related events from the patients themselves, their families and/or their affiliated hospitals. The primary endpoint was an HF-related event as a composite of cardiovascular death and hospitalization for HF decompensation. Cardiovascular death was defined as death because of myocardial infarction, ischemic stroke, congestive heart failure, or documented sudden death without apparent non-cardiovascular causes. Hospitalization for HF decompensation was diagnosed if the patient was hospitalized with typical HF symptoms and had objective signs of worsening HF that required intravenous drug administration.

Statistical analyses

Continuous values were expressed as mean±standard deviation (SD), whereas data with a skewed distribution were expressed as the median (interquartile range). We performed

Table 1. Patient Characteristics.

	All Patients (n=551)	Molar ratio of NT-proBNP/BNP		
		High molar ratio group (≥1.70) (n=275)	Low molar ratio group (<1.70) (n=276)	p value
Age (years)	72.8±12.6	74.1±12.8	71.6±12.3	0.024
Sex, male/female (male, %)	264/287 (48%)	145/130 (53%)	119/157 (43%)	0.024
Body mass index (BMI) (kg/m^2)	23.2±4.4	23.0±4.3	23.4±4.6	0.27
Hypertension (HT) (%)	393/551 (71%)	197/275 (72%)	197/276 (71%)	0.95
Diabetes mellitus (DM) (%)	135/551(25%)	71/275 (26%)	64/276 (23%)	0.47
Dyslipidemia (DLP) (%)	283/551 (51%)	131/275 (47%)	152/276 (55%)	0.080
History of cardiac disease	288/551 (52%)	164/275 (60%)	124/276 (45%)	<0.001
History of CAD	105/551 (19%)	52/275 (19%)	53/276 (19%)	0.93
History of LVH or cardiomyopathy	116/551 (21%)	75/275 (26%)	41/276 (15%)	<0.001
History of valve disease	101/551 (18%)	67/275 (24%)	34/276 (12%)	<0.001
History of AF	120/551 (22%)	77/275 (28%)	43/276 (16%)	<0.001
eGFR (mL/min/1.73 m^2)	62.8±19.9	57.5±21.5	68.2±16.6	<0.001**
Hemoglobin (Hgb) (g/dL)	13.1±1.7	12.9±1.7	13.4±1.7	0.004
Heart rate (HR) (bpm)	71±16	74±18	67±12	<0.001**
Left ventricular mass index (LVMI) (g/m^2)	83.7±19.6	86.7±20.7	80.7±17.9	0.002
LVEF (%)	61±8	59±9	62±6	<0.001
BNP and NT-proBNP				
BNP (pg/mL)	33.5 (14.3-80.4)	37.8 (15.6-111.8)	31.9 (13.6-69.5)	0.025*
NT-proBNP (pg/mL)	135 (57-433)	241 (89-755)	89 (38-207)	<0.001*
BNP (pmol/mL)	9.7 (4.1-23.2)	11.0 (4.5-32.2)	9.2 (3.9-20.1)	0.025*
NT-proBNP (pmol/mL)	15.9 (6.7-51.1)	28.4 (10.4-89.0)	10.5 (4.4-24.4)	<0.001*
Molar ratio of NT-proBNP/BNP	1.70 (1.21-2.53)	2.54 (2.09-3.41)	1.21 (0.95-1.47)	<0.001*
Medications				
ACE-I or ARB (%)	309/551 (56%)	169/275 (61%)	140/276 (51%)	0.011
Calcium-channel blockers (%)	295/551 (54%)	139/275 (51%)	156/276 (57%)	0.16
Beta blockers (%)	151/551 (27%)	79/275 (29%)	72/276 (26%)	0.49
Thiazide diuretics (%)	30/551 (5%)	14/275 (5%)	16/276 (6%)	0.72
Loop diuretics (%)	77/551 (14%)	50/275 (18%)	27/276 (10%)	0.005
Spironolactone (%)	63/551 (11%)	42/275 (15%)	21/276 (8%)	0.005
DPP IV inhibitors (%)	48/551 (9%)	26/275 (9%)	22/276 (8%)	0.54
Aspirin (%)	145/551 (26%)	76/275 (28%)	69/276 (25%)	0.48
HMG-CoA reductase inhibitors (%)	175/551 (32%)	82/275 (30%)	93/276 (34%)	0.34

Data are number of patients (%), mean±standard deviation (SD), and median (interquartile range). * Mann-Whitney U test, ** Welch's t test. CAD: coronary artery disease, LVH: left ventricular hypertrophy, AF: atrial fibrillation, eGFR: estimated glomerular filtration rate, LVEF: left ventricular ejection fraction, BNP: B-type natriuretic peptide, NT-proBNP: N-terminal pro B-type natriuretic peptide, ACE-I: angiotensin converting enzyme inhibitor, ARB: angiotensin II receptor blockers, DPP IV inhibitors: dipeptidyl peptidase IV inhibitors, HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A

a simple regression analysis to determine the correlation between the BNP and NT-proBNP levels. The frequencies of coronary risk factors and medications were compared between two groups using a chi-squared analysis. For continuous variables with equal variances, an unpaired *t*-test was performed to compare two groups. For continuous variables with unequal variances, Welch's test was performed (eGFR and heart rate in Table 1). For continuous variables with a skewed distribution, a Mann-Whitney U test was performed (BNP and NT-proBNP in Table 1). A history of cardiac diseases was defined as at least one cardiac disease [CAD, LVH or cardiomyopathy, heart valve disease (moderate to severe heart valve disease or heart valve replacement), or AF] as a comorbidity. The correlation between two continuous variables was assessed using Pearson's correlation coefficient [log (molar ratio of NT-proBNP/BNP), log BNP, log NT-proBNP, and LVMI]. Multiple linear regression analyses were used to identify the clinical factors correlated with the molar ratio of NT-proBNP/BNP, BNP levels, and NT-proBNP levels. A Cox proportional hazards regression analysis was performed to identify independent predictors of end-points (HF-related events) using the following input variables: log (molar ratio of NT-proBNP/BNP), age, gender, BMI, HT, DM, DLP, a history of cardiac disease, eGFR, hemoglobin (Hgb), heart rate (HR), and LVEF.

Survival curves for HF-related events were determined using the Kaplan-Meier method. Survival curves were compared between two or three groups with the log-rank test, as

Table 2. Molar Ratio of NT-proBNP/BNP, BNP, and NT-proBNP Levels with or without Cardiac Disease.

	With CAD (n=105)	Without CAD (n=446)	p value	With LVH/ cardiomyopathy (n=116)	Without LVH/ cardiomyopathy (n=435)	p value
BNP (pg/mL)	59.4 (24.5-128)	31.0 (13.0-73.0)	<0.001*	70.2 (30.0-156)	29.5(13.1-67.1)	<0.001*
NT-proBNP (pg/mL)	250 (87-564)	122 (56-332)	<0.001*	328 (143-946)	112 (50-298)	<0.001*
Molar ratio of NT-proBNP/BNP	1.70 (1.22-2.39)	1.70 (1.21-2.54)	0.88*	2.17 (1.51-3.09)	1.62 (1.16-2.34)	<0.001*

	With valve disease (n=101)	Without valve disease (n=450)	p value	With AF (n=120)	Without AF (n=431)	p value
BNP (pg/mL)	82.4 (33.5-165)	28.8 (12.5-66.5)	<0.001*	111 (45.0-178)	27.5 (11.9-58.4)	<0.001*
NT-proBNP (pg/mL)	417 (172-1,110)	108 (48-298)	<0.001*	565 (255-1,295)	106 (48-223)	<0.001*
Molar ratio of NT-proBNP/BNP	2.24 (1.54-3.15)	1.64 (1.16-2.33)	<0.001*	2.31 (1.40-3.46)	1.63 (1.17-2.31)	<0.001*

Data are median (interquartile range). * Mann-Whitney U test. CAD: coronary artery disease, LVH: left ventricular hypertrophy, AF: atrial fibrillation, BNP: B-type natriuretic peptide, NT-proBNP: N-terminal pro B-type natriuretic peptide.

appropriate.

Receiver operating characteristic (ROC) curves were constructed for the molar ratio of NT-proBNP/BNP, BNP, and NT-proBNP to predict HF-related events. The area under the curve (AUC) was calculated to predict HF-related events. We defined a value from the upper left corner of the ROC curve of molar ratio of NT-proBNP/BNP as the best cut-off value for the future HF-related events.

All statistical analyses were performed using the BellCurve for Excel software program, version 2.03 (Social Survey Research Information, Tokyo, Japan). Statistical significance was defined as a p value <0.05.

Results

Baseline clinical characteristics

The clinical characteristics, molar ratio of NT-proBNP/BNP, and levels of BNP and NT-proBNP in all study patients are shown in Table 1. The median values of the molar ratio of NT-proBNP/BNP, BNP, and NT-proBNP were 1.70, 33.5 pg/mL, and 135 pg/mL, respectively.

We divided all patients into two groups based on the median molar ratio of NT-proBNP/BNP (1.70). The clinical characteristics of the two groups are shown in Table 1. The patients with a high molar ratio of NT-proBNP/BNP tended to be significantly older than those with a low molar ratio. There were significantly more men in the high-molar-ratio group than in the low-molar-ratio group. There were significantly more patients with a history of cardiac disease in the high-molar-ratio group than in the low-molar-ratio group. The patients with a high molar ratio of NT-proBNP/BNP had significantly lower eGFR, Hgb, and LVEF values than those with a low molar ratio. The patients with a high molar ratio of NT-proBNP/BNP had significantly higher HR and LVMI than those with a low molar ratio. The BNP and NT-proBNP levels were significantly greater in the high-molar-ratio group than in the low-molar-ratio group. The prescription rates of ACE-I/ARB, loop diuretics, and spironolactone were significantly higher in the high-molar-ratio group than in the low-molar-ratio group. The number of patients treated with DPP-IV inhibitor was not significantly different between the high- and low-molar-ratio groups (Table 1).

The BNP and NT-proBNP levels were significantly greater in the patients with CAD, LVH/cardiomyopathy, valve disease, or AF than in those without them (Table 2). The molar ratio of NT-proBNP/BNP were significantly greater in the patients with LVH/cardiomyopathy, valve disease, or AF than in those without them. In contrast, there was no significant difference in the molar ratio of NT-proBNP/BNP between the patients with and without CAD (Table 2).

Furthermore, the LVMI was significantly correlated with the log (molar ratio of NT-proBNP/BNP), log BNP, and log NT-proBNP [log (molar ratio of NT-proBNP/BNP): r=0.14, p=0.0063; log BNP: r=0.34, p<0.001; log NT-proBNP: r=0.34, p<0.001].

Correlation between BNP and NT-proBNP

After transforming the units of BNP and NT-proBNP from pg/mL to pmol/L, the BNP levels (pmol/L) were still strongly correlated with the NT-proBNP levels (pmol/L) (r=0.924, p<0.001) (Fig. 1a). The distribution of the molar ratio of NT-proBNP/BNP was skewed with a long right tail (median: 1.70, interquartile range: 1.21-2.53) (Fig. 1b).

Multiple linear regression analyses for the molar ratio of NT-proBNP/BNP, BNP, and NT-proBNP

Multiple linear regression analyses showed that the age, sex, history of cardiac disease, eGFR, Hgb, HR, and LVEF were significantly correlated with the molar ratio of NT-proBNP/BNP (Table 3). These analyses also showed that the age, BMI, HT, history of cardiac disease, eGFR, Hgb, and LVEF were significantly correlated with the BNP levels (age: p<0.001, BMI: p=0.007, HT: p=0.003, history of cardiac disease: p<0.001, eGFR: p=0.013, Hgb: p=0.001, LVEF: p<0.001) and that the age, BMI, history of cardiac disease, eGFR, Hgb, HR, and LVEF were significantly cor-

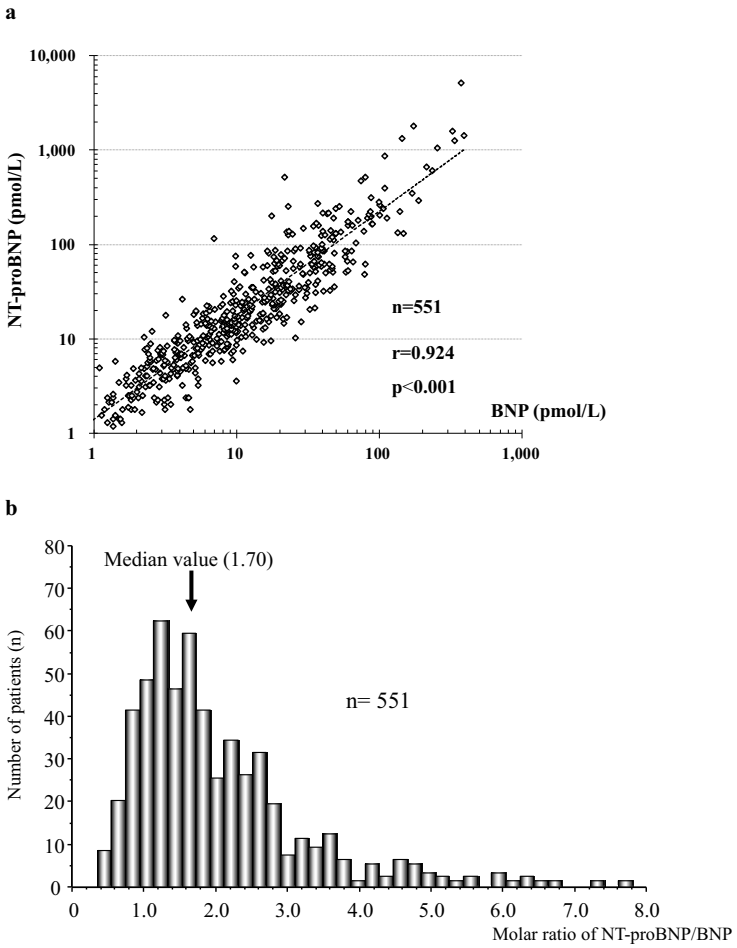

Figure 1. **(a) The correlation between the BNP and NT-proBNP levels (pmol/L). The BNP levels were strongly correlated with the NT-proBNP levels (r=0.924, p<0.001). (b) The distribution of the molar ratio of NT-proBNP/BNP. The histogram shows that the distribution of the molar ratio of NT-proBNP/BNP was skewed with a long right tail. BNP: B-type natriuretic peptide, NT-proBNP: N-terminal pro-B-type natriuretic peptide**

related with the NT-proBNP levels (age: p<0.001, BMI: p=0.007, history of cardiac disease: p<0.001, eGFR: p<0.001, Hgb: p<0.001, HR: p=0.02, LVEF: p<0.001).

The HR was significantly correlated with the molar ratio of NT-proBNP/BNP but was not significantly correlated with the BNP in a multiple linear regression analysis.

Molar ratio of NT-proBNP/BNP as a prognostic marker for HF-related events

The mean follow-up period was 487 days for all study patients. Of the 551 patients, 38 developed HF-related events during the follow-up period, including cardiovascular death (n=8) and hospitalization for HF decompensation (n=30) (Table 4). The patients with a high molar ratio of NT-proBNP/BNP had significantly more HF-related events than those with a low molar ratio. The incidence of hospitalization for HF decompensation was significantly greater in the high-molar-ratio group than in the low-molar-ratio group (Table 4). There was a gradual increase in the frequency of HF-related events with increasing molar ratio of NT-proBNP/BNP, even after dividing all patients into 3 groups based on the tertile (1.35, 2.24) of the molar ratio of NT-proBNP/BNP (Table 4).

A univariate Cox proportional hazards analysis showed that the log (molar ratio of NT-proBNP/BNP), age, BMI, DM, DLP, history of cardiac disease, eGFR, Hgb, HR, and LVEF were significantly associated with future HF-related events (Table 5). A multivariate Cox proportional hazards analysis including all of the factors that were significant in the univariate Cox proportional hazards analysis showed that the log (molar ratio of NT-proBNP/BNP) as a continuous variable was an independent predictor of future HF-related events [hazard ratio (HR): 4.42, 95% confidence interval (CI): 1.08-18.14, p=0.039] (Table 5).

A Kaplan-Meier analysis showed a significantly higher probability of primary endpoints (HF-related events) over time in the patients with a high molar ratio of NT-proBNP/BNP (≥1.70) than in those with a low molar ratio (<1.70) during the follow-up period (p<0.001) (Fig. 2a). There was a gradual increase in the frequency of HF-related events with increasing molar ratio of NT-proBNP/BNP in the Kaplan-Meier analysis, even after dividing all patients into 3

Table 3. Multiple Linear Regression Analysis to Identify Clinical Factors Correlated with Molar Ratio of NT-proBNP/BNP.

Variable	Adjusted R^2=0.325				
	Partial regression coefficient	SE	β	t	p value
Age (years)	-0.0036	0.0010	-0.1684	-3.510	<0.001
Sex, male	0.0477	0.0234	0.0921	4.144	0.042
Body mass index (kg/m^2)	-0.0015	0.0026	-0.0249	-0.563	0.57
Hypertension (HT)	0.0155	0.0241	0.0266	0.644	0.52
Diabetes mellitus (DM)	-0.0087	0.0242	-0.0144	-0.360	0.72
Dyslipidemia (DLP)	-0.0364	0.0210	0.0703	-1.738	0.08
History of cardiac disease	0.0587	0.0221	0.1122	2.654	0.008
eGFR (mL/min/1.73 m^2)	-0.0048	0.0006	0.3706	7.800	<0.001
Hemoglobin (Hgb) (g/dL)	-0.0205	0.0078	-0.1335	-2.611	0.009
Heart rate (HR) (bpm)	0.0045	0.0006	0.2858	7.175	<0.001
LVEF (%)	-0.0044	0.0014	-0.1327	-3.044	0.003

BNP: B-type natriuretic peptide, NT-proBNP: N-terminal pro B-type natriuretic peptide, eGFR: estimated glomerular filtration rate, LVEF: left ventricular ejection fraction, SE: standard error, β: standard partial regression coefficient

Table 4. HF-related Events in Patients according to Molar Ratio of NT-proBNP/BNP.

	All patients (n=551)	Median			Tertile			
		High molar ratio group (≥1.70) (n=275)	Low molar ratio group (<1.70) (n=276)	p value	Molar ratio ≥2.24 group (n=184)	1.35 ≤Molar ratio <2.24 group (n=183)	Molar ratio <1.35 group (n=184)	p value
Primary endpoint (HF-related events)	38	31	7	<0.001	26	9	3	<0.001
Cardiovascular death	8	7	1	0.074	7	1	0	0.004
Hospitalization for HF decompensation	30	24	6	0.001	19	8	3	<0.001

Data are number of patients. HF: heart failure, BNP: B-type natriuretic peptide, NT-proBNP: N-terminal pro B-type natriuretic peptide

groups based on the tertile of the molar ratio of NT-proBNP/BNP (Fig. 2b).

Additional Kaplan-Meier analyses were performed after patients were stratified into 4 groups based on the combination of the median value of the molar ratio of NT-proBNP/BNP (1.70) and BNP levels (33.5 pg/mL). In the subgroups with high BNP levels, there was a significantly higher probability of HF-related events in patients with a high molar ratio of NT-proBNP/BNP than in those with a low molar ratio (p<0.001) (Fig. 2c). Similarly, a Kaplan-Meier analysis was performed after patients were stratified into 4 groups based on the combination of the median value of the molar ratio of NT-proBNP/BNP (1.70) and NT-proBNP levels (135 pg/mL). In the subgroup with high NT-proBNP levels, there was a significantly higher probability of HF-related events in patients with a high molar ratio of NT-proBNP/BNP than in those with a low molar ratio (p=0.0090) (Fig. 2d).

ROC curve analyses to predict HF-related events

We constructed ROC curves to compare the ability of the molar ratio of NT-proBNP/BNP to predict HF-related events. The ROC curve analysis showed that the molar ratio of NT-proBNP/BNP was significantly correlated with the occurrence of HF-related events (AUC: 0.75, 95% CI: 0.67-0.83, p<0.001). The sensitivity and specificity of the median value (1.70) of the molar ratio of NT-proBNP/BNP to predict HF-related events were 82%, and 52%, respectively. The sensitivity and specificity of the nearest value (2.05) of the molar ratio of NT-proBNP/BNP from the upper left corner of the ROC curve to predict HF-related events were 79%, and 64%, respectively. Using this cut-off value (2.05) of the molar ratio of NT-proBNP/BNP, a multivariate Cox proportional hazards analysis showed that the higher molar ratio of NT-proBNP/BNP (≥2.05) was a significant and independent predictor for the HF-related events (HR: 2.65, 95% CI: 1.13-6.19, p=0.025). ROC curve analyses showed that BNP and NT-proBNP level was significantly correlated with the occurrence of HF-related events, respectively (BNP, AUC: 0.84, 95% CI: 0.78-0.90, p<0.001; NT-proBNP, AUC: 0.88, 95% CI: 0.83-0.92, p<0.001). There was no significant difference in the AUC between molar ratio of NT-proBNP/BNP and BNP for the prediction of HF-related events (p=0.086). The AUC of the molar ratio of NT-proBNP/BNP for the prediction of HF-related events was significantly lower than that of NT-proBNP (p=0.0013).

Table 5. Univariate and Multivariate Cox Proportional Hazards Analysis to Identify Predictors of Primary Endpoints (HF-related Events).

Variable	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	p value	HR (95% CI)	p value
Age (years)	1.07 (1.03-1.11)	<0.01	1.05 (1.01-1.10)	0.030
Sex, male	0.86 (0.45-1.64)	0.65	-	
Body mass index (kg/m^2)	0.91 (0.84-0.98)	0.016	0.96 (0.90-1.03)	0.28
Hypertension (HT)	0.77 (0.38-1.55)	0.46	-	
Diabetes mellitus (DM)	2.27 (1.18-4.37)	0.014	3.04 (1.45-6.38)	0.003
Dyslipidemia (DLP)	0.35 (0.32-0.70)	<0.01	0.54 (0.25-1.19)	0.13
History of cardiac disease	19.9 (4.78-82.77)	<0.001	11.23 (2.54-49.65)	0.001
eGFR (mL/min/1.73 m^2)	0.94 (0.93-0.96)	<0.001	0.96 (0.94-0.99)	0.003
Hemoglobin (Hgb) (g/dL)	0.70 (0.58-0.84)	<0.001	1.10 (0.89-1.34)	0.37
Heart rate (HR) (bpm)	1.03 (1.01-1.05)	0.001	1.01 (0.99-1.03)	0.35
LVEF (%)	0.92 (0.90-0.94)	<0.001	0.98 (0.95-1.02)	0.36
Log (Molar ratio of NT-proBNP/BNP)	24.0 (9.32-61.72)	<0.001	4.42 (1.08-18.14)	0.039

HF: heart failure, eGFR: estimated glomerular filtration rate, LVEF: left ventricular ejection fraction, BNP: B-type natriuretic peptide, NT-proBNP: N-terminal pro B-type natriuretic peptide, HR: hazard ratio, CI: confidence interval

Discussion

In the present study, the BNP and NT-proBNP levels were significantly higher in patients with a high molar ratio of NT-proBNP/BNP (≥1.70) than in those with a low molar ratio (<1.70). Multiple linear regression analyses showed that the age, sex, history of cardiac diseases, eGFR, Hgb, HR, and LVEF were significantly correlated with molar ratio of NT-proBNP/BNP. Stable outpatients with a high molar ratio of NT-proBNP/BNP developed HF-related events significantly more frequently during the follow-up period than those with low molar ratio. The best cut-off value of the molar ratio of NT-proBNP/BNP for predicting HF-related events was 2.05 in stable outpatients with cardiovascular risk factors (sensitivity: 79%, specificity: 64%). In patients with elevated BNP or NT-proBNP levels, the molar ratio of NT-proBNP/BNP can provide useful prognostic information for HF management.

Theoretically, BNP and NT-proBNP secreted from cardiomyocytes should have a molar ratio of 1.00; however, our study showed that the molar ratio of NT-proBNP/BNP was highly variable (Fig. 1b). The deviation from 1.00 may be due to differences in the biological characteristics of BNP and NT-proBNP. In the systemic circulation, BNP mediates a variety of biological effects via interaction with natriuretic peptide receptor type A (NPR-A), causing intracellular cGMP production. The physiological effects of BNP include natriuresis/diuresis, peripheral vasodilatation, and the inhibition of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system. Furthermore, BNP is cleared from the plasma by binding to NPR-C and through proteolysis by NEP. In contrast, NT-proBNP does not bind to NPR-A or NPR-C and is not degraded by NEP.

NT-proBNP is cleared from the circulation more slowly than BNP (half-life of 120 vs. 20 minutes) (9, 12). The influence of the renal function on NT-proBNP seems to be greater than on BNP (13). The range of NT-proBNP levels has been shown to be wider than that of BNP levels with the same severity of HF based on their New York Heart Association (NYHA) classification (9-11). Previous studies have reported that the activation of NEP may be associated with exacerbated HF and sympathetic nervous system and kidney disease (15-19), suggesting that the enhanced degradation of the bioactive BNP by NEP may cause a rapid decrease in the plasma BNP levels compared to stable levels of NT-proBNP in the blood stream. These different regulatory mechanisms suggest that the molar ratio of NT-proBNP/BNP may be greater under conditions of HF with elevated NEP activity. In the present study, the patients with a high molar ratio of NT-proBNP/BNP were significantly older and had significantly lower eGFR, Hgb, and LVEF than those with a low molar ratio. The patients with a high molar ratio of NT-proBNP/BNP also had significantly higher HR and LVMI values than those with a low molar ratio. Significantly more patients had a history of cardiac disease in the high-molar-ratio group than in the low-molar-ratio group (Table 1). The molar ratio of NT-proBNP/BNP may reflect a condition at risk of HF, based on the age, renal function, anemia, and cardiac function, in patients with cardiovascular risk factors.

In the present study, we found that the HR was significantly correlated with the molar ratio of NT-proBNP/BNP in stable outpatients with cardiovascular risk factors (Table 3). However, the HR was not significantly correlated with the BNP levels. The resting HR reflects the autonomic activity and is correlated with hyperglycemia, hyperinsulinemia, metabolic syndrome, and mortality (20). Autonomic imbalance has been associated with increased cardiac mortality. An elevated resting HR was associated with an increased risk of HF (21). As such, the molar ratio of NT-proBNP/BNP may reflect the autonomic activity and major cardiac

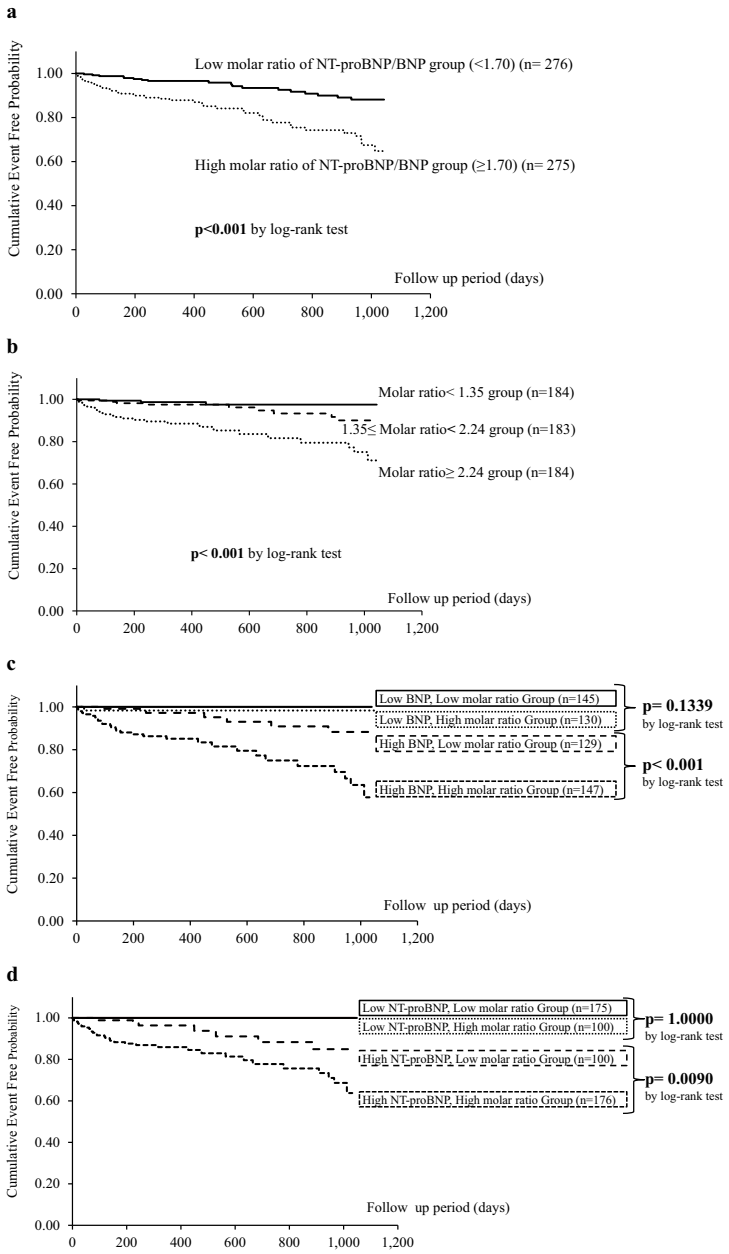

Figure 2. **A Kaplan-Meier analysis for the probability of HF-related events. (a) A Kaplan-Meier analysis based on the median value (1.70) of the molar ratio of NT-proBNP/BNP showed a significantly higher probability of HF-related events in the patients with a high molar ratio of NT-proBNP/BNP (≥1.70) than in those with a low molar ratio (<1.70) during the follow-up period. (b) A Kaplan-Meier analysis based on the tertile (1.35, 2.24) of the molar ratio of NT-proBNP/BNP showed that there was a gradual increase in the frequency of HF-related events with the increasing molar ratio of NT-proBNP/BNP. (c) A Kaplan-Meier analysis for the probability of HF-related events in subgroups stratified by the molar ratio of NT-proBNP/BNP and BNP levels. Patients were stratified into 4 subgroups based on the combination of the median value of the molar ratio of NT-proBNP/BNP (1.70) and BNP levels (33.5 pg/mL). In the subgroups with high BNP levels, patients with a high molar ratio of NT-proBNP/BNP had a significantly higher probability of HF-related events than those with a low molar ratio. (d) A Kaplan-Meier analysis for the probability of HF-related events in subgroups stratified by the molar ratio of NT-proBNP/BNP and NT-proBNP levels. Patients were stratified into 4 subgroups based on the combination of the median value of the molar ratio of NT-proBNP/BNP (1.70) and NT-proBNP levels (135 pg/mL). In the subgroups with high NT-proBNP levels, patients with a high molar ratio of NT-proBNP/BNP had a significantly higher probability of HF-related events than those with a low molar ratio. HF: heart failure, BNP: B-type natriuretic peptide, NT-proBNP: N-terminal pro B-type natriuretic peptide**

conditions associated with an elevated HR.

We proposed a cut-off value of 2.05 for the molar ratio of NT-proBNP/BNP to predict HF-related events in stable outpatients with cardiovascular risk factors. This value was equivalent to 5.0 for the plasma concentration ratio of NT-proBNP (pg/mL)/BNP (pg/mL).

Few studies have measured the BNP and NT-proBNP levels simultaneously in humans (9-11). Although BNP and NT-proBNP should be secreted from cardiomyocytes in response to increased myocardial wall stress in a molar ratio of 1.00, the calculated molar ratio of NT-proBNP/BNP is often >1.00 in routine clinical practice. The clinical significance of this phenomenon is still unknown. In the present study, we measured the BNP and NT-proBNP levels simultaneously in 551 stable outpatients with cardiovascular risk factors and examined the clinical impact of these levels on the prognostic value of the molar ratio of NT-proBNP/BNP. Our findings showed that the molar ratio of NT-proBNP/BNP provided significant prognostic information for stable outpatients with cardiovascular risk factors. However, the AUC of the ROC curve analysis with the molar ratio of NT-proBNP/BNP for the prediction of HF-related events was not significantly greater than that of BNP or NT-proBNP in the present study.

Among the patients with elevated BNP or NT-proBNP levels in the present study, those with a high molar ratio of NT-proBNP/BNP had a significantly higher probability of HF-related events than those with a low molar ratio (Fig. 2c and d). These results suggest that the molar ratio of NT-proBNP/BNP may help identify outpatients at high risk for HF-related events.

Previous studies have suggested that dipeptidyl-peptidase IV (DPP-IV) inhibitors, an antidiabetic drug, may affect the secretion system of natriuretic peptide, such as the molar ratio of NT-proBNP/BNP, since DPP-IVs cleave BNP_{1-32} to BNP_{3-32} (22, 23). In the present study, the number of patients treated with DPP-IV inhibitors was not significantly different between the two molar ratio groups (Table 1). However, the secretion system of natriuretic peptide from the human heart may be complex. As such, further studies regarding how DPP-IV inhibitors affect the natriuretic peptide system in humans are needed.

The present study has several limitations. First, it was a single-center study with a relatively small sample size. Second, several studies have reported that not only BNP and NT-proBNP but also proBNP are secreted in the blood stream, with their values increased in the peripheral blood of patients with HF (9, 24-27). The current commercial assay kits for measuring BNP (Shionoria BNP; Shionogi) and NT-proBNP (Roche Diagnostics) may not be specific for just BNP or NT-proBNP, possibly cross-reacting with proBNP (28, 29). Third, the present study was observational (prospective cohort study), and the patients received no specific intervention or therapy. Further analyses are needed in a larger and independent population.

In summary, the HR was significantly correlated with the molar ratio of NT-proBNP/BNP, but not with BNP levels, in stable outpatients with cardiovascular risk factors. A high molar ratio of NT-proBNP/BNP was associated with future HF-related events, and we proposed a cut-off value of 2.05 for the molar ratio of NT-proBNP/BNP for HF-related events in stable outpatients with cardiovascular risk factors.

The authors state that they have no Conflict of Interest (COI).

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