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ORIGINAL ARTICLE
Myocardial Disease

Soluble Neprilysin
— Cardiac Function and Outcome in Hypertrophic Cardiomyopathy —

Akiomi Yoshihisa, MD, PhD; Tetsuro Yokokawa, MD, PhD; Yasuhiro Ichijo, MD; Yusuke Kimishima, MD; Yuki Kanno, MD; Tomofumi Misaka, MD, PhD; Takamasa Sato, MD, PhD; Masayoshi Oikawa, MD, PhD; Atsushi Kobayashi, MD, PhD; Takayoshi Yamaki, MD, PhD; Koichi Sugimoto, MD, PhD; Hiroyuki Kunii, MD, PhD; Yasuchika Takeishi, MD, PhD

Background: Circulating soluble neprilysin (sNEP) predicts outcome in heart failure (HF) patients with reduced ejection fraction (EF), but not in those with preserved EF. We examined sNEP in patients with hypertrophic cardiomyopathy (HCM), and their correlations with other biomarkers, cardiac function, and clinical outcome.

Methods and Results: We examined the associations between sNEP and the laboratory and echocardiography parameters in the HCM patients (n=93). Regarding the laboratory data, sNEP had a significant positive correlation with B-type natriuretic peptide (BNP; R=0.326, P=0.003), but not with troponin I. As for the echocardiographic parameters, sNEP negatively correlated with left ventricular EF (R=−0.283, P=0.009) and right ventricular fractional area change (R=−0.277, P=0.012), but not with left ventricular mass. Next, we prospectively followed up on the patients for cardiac events, including worsening HF or cardiac death, and all-cause mortality. On Kaplan-Meier analysis (mean follow-up, 1,021 days), the cardiac event rate and all-cause mortality were similar between the higher sNEP group (sNEP ≥median level of 1.43 ng/mL, n=46) and lower sNEP group (sNEP <1.43 ng/mL, n=47). On Cox proportional hazard analysis, sNEP was not a predictor of cardiac event or all-cause mortality.

Conclusions: Soluble neprilysin appears to correlate with BNP and cardiac systolic function, but it is not significantly associated with prognosis in HCM patients.

Key Words: Echocardiography; Hemodynamics; Hypertrophic cardiomyopathy; Natriuretic peptide; Neprilysin

Hypertrophic cardiomyopathy (HCM) is defined as the presence of increased left ventricular (LV) wall thickness or fibrosis that is not solely explained by abnormal loading conditions.[1–3] Neprilysin, also known as neutral endopeptidase (NEP), is a membrane-bound enzyme that breaks down numerous vasoactive peptides.[4,5] It was recently the focus of the Prospective comparison of ARNi (NEP inhibitor) with Angiotensin-Converting Enzyme Inhibitor to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial in patients with heart failure (HF).[6–10]

NEP is mainly expressed in the kidneys, but it is also present in the lungs, endothelial cells, vascular smooth muscle cells, cardiac myocytes, fibroblasts, neutrophils, adipocytes, testes, and brain, with the highest concentration in the proximal tubules of nephrons.[11] To date, the mechanism of action of NEP is complex and ubiquitous, and remains poorly understood.[4,12,13] NEP substrates that include peripheral vasodilation include natriuretic peptides, bradykinin, substance P and adrenomedulin, and those with peripheral vasoconstriction include angiotensin II and endothelin-1.[4] The net effects of NEP on vascular tone will depend on whether the predominant degraded subjects are vasodilators or vasoconstrictors.[4] Circulating soluble NEP (sNEP) and sNEP activity are modestly correlated; thus, circulating sNEP is biologically active in HF patients.[14]

The associations between circulating sNEP and prognosis in HF patients are controversial.[5,12,15–18] sNEP predicts outcomes in HF patients with reduced ejection fraction (HFrEF) or acute decompensated HF,[5,12,15–17] but it is not associated with prognosis in HF patients with preserved ejection fraction (HFpEF).[18] In such HFpEF patients, no significant correlation has been reported between sNEP and LV filling pressure on right-heart

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Department of Cardiovascular Medicine (A.Y., T. Yokokawa, Y.I., Y. Kimishima, Y. Kanno, T.M., T.S., M.O., A.K., T. Yamaki, K.S., H.K., Y.T.), Department of Advanced Cardiac Therapeutics (A.Y., T.M.), Department of Pulmonary Hypertension (T. Yokokawa, K.S.), Fukushima Medical University, Fukushima, Japan

Mailing address: Akiomi Yoshihisa, MD, PhD, Department of Cardiovascular Medicine, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, Japan. E-mail: yoshihis@fmu.ac.jp

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catheterization (RHC), and between sNEP and fibrosis on cardiac magnetic resonance imaging (CMR) or myocardial biopsy.[18] Inhibiting NEP augments the naturally occurring natriuretic peptides, which promote natriuresis, induce vasodilation, and reduce cardiac hypertrophy and fibrosis.[12] There have been no reported data, however, on the association between sNEP and cardiac function in patients with HCM.

Therefore, in the current study, we investigated the relationships between sNEP and other biomarkers (e.g., natriuretic peptide, cardiac troponins), cardiac function (e.g. echocardiography, RHC), and prognosis in HCM patients.

Methods

Subjects and Study Protocol

This was a cross-sectional and prospective observational study that enrolled 93 consecutive patients with HCM who were comprehensively examined and diagnosed according to the current guidelines[1–3] at Fukushima Medical University Hospital between 2011 and 2016. None of the patients had been taking NEP inhibitors. After overnight fasting, blood samples were obtained from each patient in a stable condition at discharge or at the outpatient setting in the morning (08:00–11:00 hours). All samples were frozen and stored in aliquots at −80°C. Circulating plasma sNEP was measured on radioimmunoassay (ELH-Neprilysin-1 kit; RayBiotech, Norcross, GA, USA).

We first examined the associations between sNEP and the laboratory, echocardiography, and RHC parameters in HCM patients. Second, the patients (n=93) were divided into 2 groups based on median sNEP: the low group, sNEP <1.43 ng/mL (n=47); and the high group, sNEP ≥1.43 ng/mL (n=46). These patients were followed up until 2018 for cardiac events and all-cause death; we were able to follow up on all patients. Cardiac events were defined as worsened HF or cardiac death. Cardiac death was classified by independent experienced cardiologists as worsened HF in accordance with the Framingham criteria, ventricular fibrillation documented on electrocardiogram or implantable devices, or sudden death. Status and dates of death were obtained from the patient medical records. If these data were unavailable, patient status was ascertained by a telephone call to the patient's referring hospital physician. Those carrying out the survey were blind to the analyses, and written informed consent was obtained from all subjects. The study protocol was approved by the Ethics Committee of Fukushima Medical University, and was carried out in accordance with the principles outlined in the Declaration of Helsinki. Reporting of the study conforms to STROBE, along with references to STROBE and the broader EQUATOR guidelines.[19]

Laboratory Data

Circulating plasma sNEP was measured on radioimmunoassay (ELH-Neprilysin-1 kit, RayBiotech). N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured using an electrochemiluminescence immunoassay (Elecsys pro BNP II; Roche Diagnostics, Rotkreuz, Switzerland),

Table. Patient Characteristics vs. sNEP Status (n=93)

	sNEP (Low vs. High)			Correlation with sNEP	
	Low sNEP (sNEP <1.43, n=47)	High sNEP (sNEP ≥1.43, n=46)	P-value	Correlation coefficient	P-value
Neprilysin (ng/mL)	0.84 (0.74–1.13)	2.46 (1.87–3.48)	<0.001		
Demographics					
Age (years)	64.3±13.1	61.6±14.6	0.341	0.015	0.883
Male	31 (66.0)	31 (67.4)	0.883	0.028	0.791
BMI (kg/m^2)	24.4±4.0	24.3±3.7	0.936	0.015	0.891
HCM type					
APH	12 (25.5)	12 (26.1)	0.951	−0.108	0.305
HOCM	11 (23.4)	10 (21.7)	0.848	−0.028	0.786
D-HCM	3 (6.4)	8 (17.4)	0.100	0.237	0.022
Others	21 (44.7)	16 (34.8)	0.332	−0.039	0.709
Medications					
RAA inhibitor	24 (48.9)	35 (76.1)	0.012	0.212	0.041
β-blocker	43 (91.5)	41 (89.1)	0.700	0.078	0.458
Diuretic	16 (34.0)	21 (45.7)	0.253	0.258	0.012
MRA	11 (23.4)	12 (26.1)	0.764	0.172	0.099
Laboratory data					
Hemoglobin (g/dL)	13.6±1.8	14.1±1.7	0.263	0.104	0.338
NT-proBNP (pg/mL)	768.0 (343.5–2,511.0)	1,024.5 (297.5–3,033.5)	0.234	0.182	0.080
BNP (pg/mL)	121.7 (62.7–373.1)	215.7 (97.9–566.2)	0.084	0.489	<0.001
Creatinine (mg/dL)	1.0±0.3	1.0±0.4	0.776	−0.022	0.840
GFR (mL/min/1.73 cm^2)	59.8±17.8	59.5±18.8	0.946	0.029	0.806
Sodium (mEq/L)	140.1±2.5	140.3±1.9	0.721	0.110	0.311
CRP (mg/dL)	0.05 (0.03–0.12)	0.14 (0.04–0.40)	0.319	0.196	0.077
Troponin I (ng/mL)	0.05 (0.03–0.39)	0.04 (0.03–0.19)	0.488	0.173	0.129
Troponin T (ng/mL)	0.015 (0.007–0.046)	0.030 (0.011–0.071)	0.272	−0.046	0.825

(**Table** continued the next page.)

	sNEP (Low vs. High)			Correlation with sNEP	
	Low sNEP (sNEP <1.43, n=47)	High sNEP (sNEP ≥1.43, n=46)	P-value	Correlation coefficient	P-value
Echocardiography					
LVEF (%)	62.4±10.0	55.8±16.5	0.032	–0.283	0.009
IVSWT (mm)	15.2±5.0	15.1±4.0	0.932	–0.095	0.382
LVDD (mm)	44.0±9.0	46.9±11.6	0.192	0.202	0.062
LVSD (mm)	27.3±9.9	32.2±13.8	0.059	0.225	0.037
PWT (mm)	12.9±3.2	11.9±2.4	0.111	–0.160	0.141
MWT (mm)	21.8±3.9	20.6±4.3	0.184	–0.188	0.071
LV mass (g)	245.1±91.0	252.7±87.5	0.694	0.049	0.654
LVMI (g/m^2)	144.6±51.8	151.1±47.7	0.550	0.048	0.666
LAV (mL)	66.5±43.4	75.5±40.2	0.343	0.222	0.049
LVOT-PG (mmHg)	32.9±12.8	32.0±11.9	0.824	0.072	0.792
Mitral valve E/e'	13.4±5.6	14.4±6.8	0.436	0.179	0.115
RA end-systolic area (cm^2)	15.1±5.1	15.2±5.5	0.920	0.164	0.167
RVA diastole (cm^2)	15.5±6.1	14.4±4.3	0.480	–0.005	0.971
RVA systole (cm^2)	8.3±3.8	8.7±3.5	0.728	0.103	0.496
RV-FAC (%)	47.0±11.9	40.1±11.4	0.053	–0.277	0.012
IVC diameter (mm)	13.3±3.0	14.2±3.6	0.195	0.203	0.071
TRPG (mmHg)	27.3±10.5	26.6±13.1	0.812	0.061	0.649
Tricuspid valve S' (cm)	11.0±3.6	8.8±2.9	0.053	–0.101	0.558
RHC (n=63)					
mPAP (mmHg)	18.5±4.9	21.7±8.2	0.058	0.202	0.110
sPAP (mmHg)	29.5±6.8	33.0±11.1	0.126	0.131	0.301
dPAP (mmHg)	12.1±3.9	15.2±6.8	0.034	0.264	0.035
RAP (mmHg)	5.9±2.4	7.1±3.7	0.115	0.203	0.095
PAWP (mmHg)	12.2±4.3	14.5±7.9	0.148	0.128	0.315
Cardiac output (L/min)	4.3±1.4	4.2±1.0	0.551	–0.120	0.347
Cardiac index (L/min/m^2)	2.6±0.7	2.5±0.5	0.604	–0.161	0.204

Data given as median (IQR), n (%) and mean±SD. APH, apical hypertrophy; BMI, body mass index; BNP, B-type natriuretic peptide; CRP, C-reactive protein; D-HCM, dilated phase hypertrophic cardiomyopathy; dPAP, diastolic pulmonary artery pressure; E/e', ratio of the peak transmitral velocity during early diastole to the peak mitral valve annular velocity during early diastole; GFR, glomerular filtration rate; HCM, hypertrophic cardiomyopathy; HOCM, hypertrophic obstructive cardiomyopathy; IVC, inferior vena cava; IVSWT, interventricular septum wall thickness; LAV, left atrial volume; LV, left ventricular; LVDD, left ventricular diastolic diameter; LVOT-PG, left ventricular outflow tract pressure gradient; LVSD, left ventricular systolic diameter; mPAP, mean pulmonary artery pressure; MRA, mineralocorticoid receptor antagonist; MWT, maximum wall thickness; NYHA, New York Heart Association; PAWP, pulmonary artery wedge pressure; PWT, posterior wall thickness; RA, right atrial; RAA, renin-angiotensin-aldosterone; RAP, right atrial pressure; RHC, right heart catheterization; RV, right ventricle; RVA, right ventricular area; RV-FAC, right ventricular fractional area change; sNEP, soluble neprilysin; sPAP, systolic pulmonary artery pressure; TRPG, tricuspid regurgitation pressure gradient.

and B-type natriuretic peptide (BNP) was measured using a specific immunoradiometric assay (Shionoria BNP kit, Shionogi, Osaka, Japan). High-sensitivity cardiac troponin I was measured in EDTA anticoagulated plasma using a refined assay (Abbott-Architect, Abbott Laboratories, Abbott Park, IL, USA). High-sensitivity cardiac troponin T was also measured using an electrochemiluminescence immunoassay (Elecsys Troponin T, Roche Diagnostics).[20] These assays were performed in a blinded manner by experienced laboratory technicians.

Echocardiography

Echocardiography was performed in a blinded manner by experienced echocardiographers using standard techniques.[21–23] Echocardiography parameters included LV ejection fraction (LVEF), LV diastolic diameter, LV systolic diameter (LVSD), interventricular septum wall thickness, posterior wall thickness, maximum wall thickness, LV mass, LV mass index (LVMI), left atrium volume, right atrium and ventricle dimensions and areas, right ventricular fractional area change (RV-FAC), inferior vena cava diameter, tricuspid regurgitation pressure gradient (TRPG), and tissue Doppler-derived tricuspid lateral annular systolic velocity (tricuspid valve S').[21–23] LVEF was calculated using Simpson's method. RV-FAC, defined as (end diastolic area–end systolic area)/end diastolic area×100, was a measure of RV systolic function.[22] LV mass and LVMI were determined as previously reported.[21] All measurements were performed using ultrasound systems (ACUSON Sequoia, Siemens Medical Solutions USA, Mountain View, CA, USA).

RHC and Hemodynamics

All RHC were performed with the patients in a stable condition, in a resting supine position under fluoroscopy guidance, at room air, and at rest for >30 min after catheter placement. Pulmonary artery pressure (PAP), pulmonary artery wedge pressure (PAWP), right atrial pressure, and cardiac output were measured using a 7F Swan-Ganz catheter (Edwards Lifesciences, Irvine, CA, USA).[24]

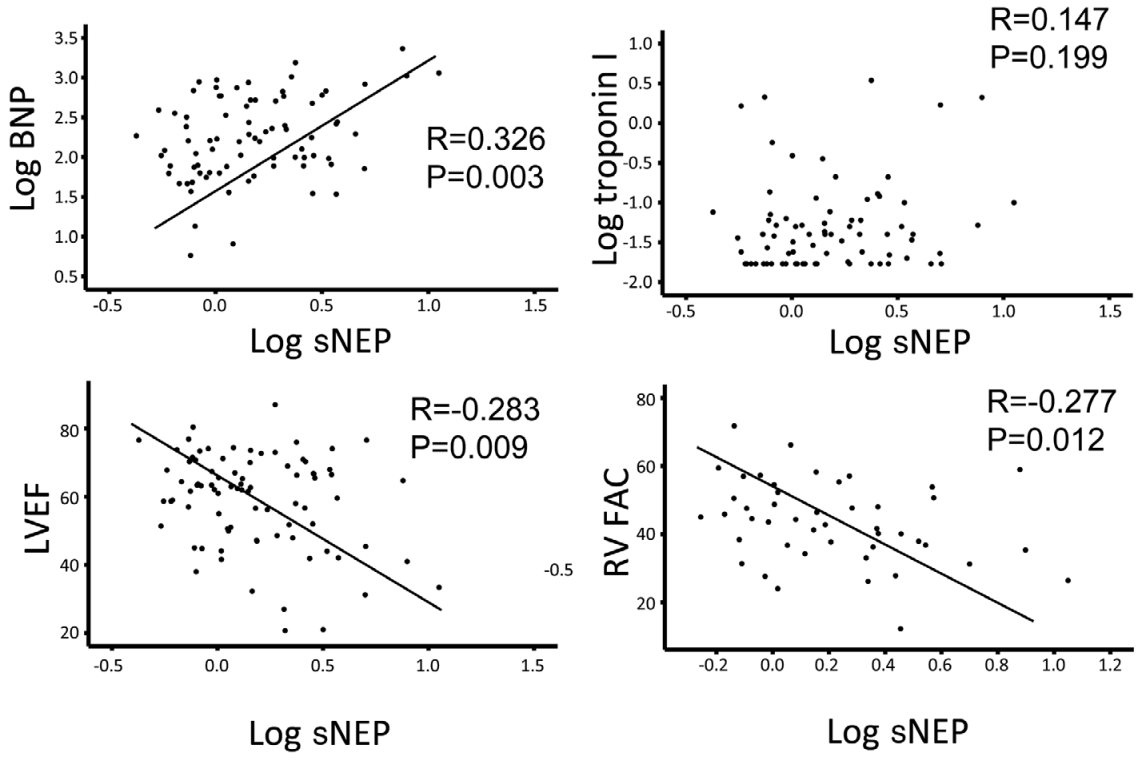

Figure 1. Log soluble neprilysin (log sNEP) vs. log B-type natriuretic peptide (logBNP), log troponin I, left ventricular ejection fraction (LVEF) and right ventricular fractional area change (RV-FAC).

Statistical Analysis

Normally distributed data are presented as mean±SD, and non-normally distributed data are presented as median (IQR). The categorical variables are expressed as numbers and percentages, and the chi-squared test was used for comparison. Parametric variables were compared using Student's t-test, and non-parametric variables were compared using the Mann-Whitney U-test. Correlations between sNEP and laboratory, echocardiography, and RHC parameters were assessed using Pearson's correlation analysis for parametric variables and Spearman's correlation analysis for non-parametric variables, as well as logistic regression analysis. Kaplan-Meier analysis with log-rank test was used to assess cardiac event rate or all-cause mortality. The Kaplan-Meier survival curves for the 2 groups were plotted against the time to follow-up period. These curves help in identifying non-proportionality patterns in hazard functions such as convergence (difference in risk between the 3 groups decreases with time), divergence, or crossing of the curves. In addition, the Schoenfeld test for violation of proportional hazards, which assesses the correlation between scaled residuals and time, was also conducted. Cox proportional hazard analysis was used to evaluate high sNEP (categorical variable) and sNEP levels (continuous variable) as predictors of cardiac event and all-cause mortality. $P<0.05$ was considered statistically significant for all comparisons. These analyses were performed using SPSS ver. 24.0 (IBM, Armonk, NY, USA).

Results

Subject clinical features are listed in **Table**. Use of renin-angiotensin-aldosterone (RAA) inhibitor was higher in the high sNEP group than in the low sNEP group. In contrast, age, sex, body mass index, type of HCM and other medications were similar between the groups. With regard to the laboratory data, BNP tended to be higher in the high sNEP group than in the low sNEP group. In addition, compared with the low sNEP group, regarding the echocardiography and RHC parameters, LVEF was significantly lower, LVSD tended to be higher, RV-FAC and tricuspid valve S' tended to be lower, and diastolic PAP was significantly higher in the high sNEP group. Other parameters were comparable between the groups.

Next, we focused on the correlations among sNEP and BNP, troponin I, LVEF and RV-FAC. As shown in **Figure 1**, there were significant correlations between sNEP and log BNP ($R=0.326$, $P=0.003$), LVEF ($R=-0.288$, $P=0.009$) and RV-FAC ($R=-0.277$, $P=0.012$), but not with troponin I. Similar data except dilated phase HCM are shown in **Supplementary Figure 1**. In addition, the logistic regression analysis between sNEP and other parameters is presented in **Table**. There were significant correlations between sNEP and the presence of dilated phase HCM (coefficient, 0.237; $P=0.022$), use of RAA inhibitor (coefficient, 0.212; $P=0.041$), diuretics (coefficient, 0.258; $P=0.012$), BNP (coefficient, 0.489; $P<0.001$), LVEF (coefficient, −0.288; $P=0.008$), LVSD (coefficient, 0.225; $P=0.037$), left atrial volume (coefficient, 0.222; $P=0.049$)

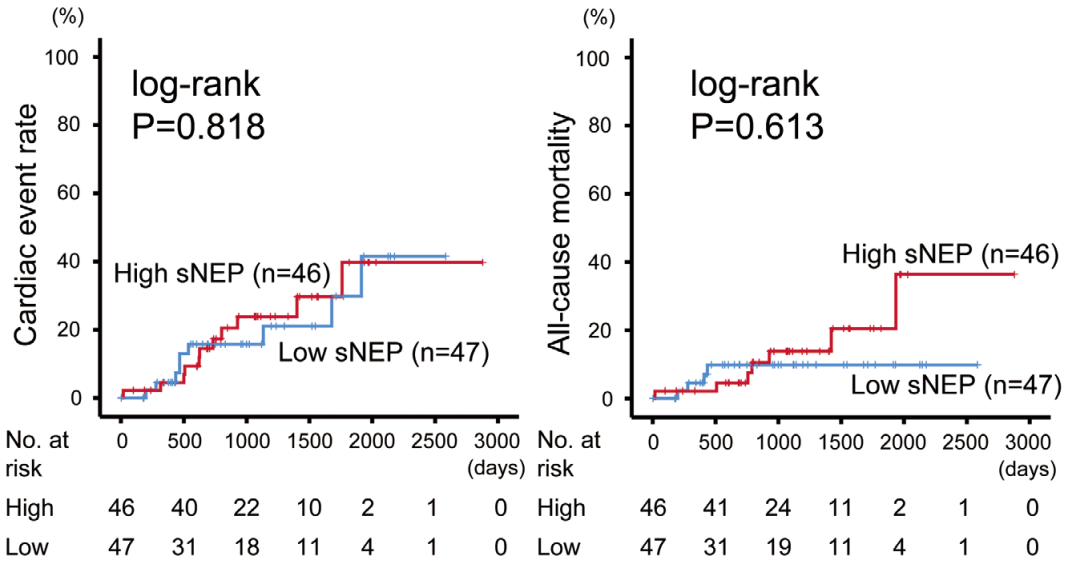

Figure 2. Kaplan-Meier analysis for cardiac event rate and all-cause mortality between the low and high soluble neprilysin (sNEP) groups.

and diastolic PAP (coefficient, 0.264; P=0.035), but not with other parameters. On multiple logistic regression analysis, of these confounding factors, BNP was the only independent factor associated with circulating sNEP (coefficient, 0.530; P=0.008).

In the follow-up period (mean, 1,021±616 days; range, 15–2,878 days), of the 93 enrolled patients, 20 had cardiac events, consisting of 7 cardiac deaths (4 ventricular fibrillation, 2 HF and 1 sudden death) and 13 cases of worsening HF, and there were 11 all-cause deaths. On Kaplan-Meier analysis (**Figure 2**), cardiac event rate and all-cause mortality were similar between the high and low sNEP groups (log-rank P=0.818, log-rank P=0.613, respectively). Similar data except dilated phase HCM are shown in **Supplementary Figure 2**. On Cox proportional hazard analysis, neither sNEP level (as a continuous variable: HR, 0.931; 95% CI: 0.695–1.248, P=0.633) nor high sNEP (as a categorical variable: HR, 1.109; 95% CI: 0.459–2.682, P=0.818) were predictors of cardiac events. In addition, neither sNEP (as a continuous variable: HR, 1.194; 95% CI: 0.972–1.468, P=0.092) nor high sNEP (as a categorical variable: HR, 1.507; 95% CI: 0.440–5.158, P=0.513) were predictors of all-cause mortality.

Discussion

To the best of our knowledge, the present study is the first to report that sNEP correlates with BNP, LVEF and RV-FAC, but that it is not significantly associated with prognosis in HCM patients.

The production of soluble/non-membrane-associated counterparts of membrane-bound proteins has been studied extensively. This is known to occur as a consequence of ectodomain shedding, which involves the proteolytic cleavage of the extracellular domain, or the release of non-membrane-associated enzyme from cells via exosomes. Catalytically active NEP has been detected in both the media of lymphoblastoid cell lines[25] and in serum from coal miners exposed to coal dust.[26] sNEP has also been detected in sera from patients with HF.[14]

No relationship has yet been found between sNEP concentration and either BNP or NT-proBNP in HF patients.[5,12,16,17] Unlike previously reported findings,[5,12,16,17] in the current study, we found correlations between sNEP and BNP, but not with NT-proBNP, which is not a substrate for NEP. NEP activity varies in acute and chronic HF as a function of circulating BNP.[27] The higher the circulating BNP (>916 pg/mL), the lower the activity of the circulating NEP, and vice versa.[27] This suggests that BNP, a substrate of NEP, may act as an endogenous inhibitor of circulating NEP.[28] To the best of our knowledge, the present study is the first to report the correlations between sNEP and BNP, LVEF, and RV-FAC in HCM patients.

Consistent with a previous report on patients with HFpEF,[18] no relationship was observed between sNEP and the RHC parameters, including cardiac index and PAWP. In a study on HFpEF patients by Goliasch et al, there was no significant correlation between sNEP and LV filling pressures, RV systolic function or fibrosis, on CMR or myocardial biopsy.[18] Regarding right heart function, concordant with the present study, we recently reported that sNEP tended to correlate with RV-FAC in patients with pulmonary hypertension.[29] As well as the hemodynamic parameters, shear stress, and volume overload, NEP expression and release are also reported to be regulated by inflammation.[30] These factors may contribute to an association between sNEP and RV-FAC. The current study is the first to present correlations between sNEP and BNP, LVEF, and RV-FAC in HCM patients.

Circulating NEP and prognosis in HF patients are controversial.[5,12,15–18] Bayes-Genis et al demonstrated a positive association between circulating NEP and cardiovascular prognosis, particularly cardiovascular mortality and HF hospitalization, in patients with chronic HF who were

followed for a mean of 4.1 years.[12] High circulating NEP is associated with cardiovascular death or HF hospitalization, independently from NT-proBNP.[12] Nunez et al reported that sNEP predicted an increased risk of recurrent all-cause, cardiovascular, and HF admissions during a mean 3.4-year follow-up, in patients with acute HF.[15] Conversely, in a recent observational, non-interventional registry of 144 patients with HFpEF, Goliasch et al could not confirm the associations between NEP and cardiovascular mortality or hospitalization for worsening HF, in contrast to HFrEF patients.[18] sNEP in HFpEF is 3-fold higher than in HFrEF.[18] This mismatch between sNEP and its target protein levels might explain the lack of correlation between sNEP and prognosis/functional measures in HFpEF.[18] Given that there are differences in the prognostic utility of biomarkers between HFrEF vs. HFpEF,[31,32] this difference may affect the lack of prognostic impact of sNEP on the present HCM patients. In addition, sNEP changes in HF with hemodynamics are controversial.[33,34] Takahama et al recently reported that sNEP concentration did not change between admission and just before discharge.[34] Conversely, Arrigo et al recently reported that sNEP concentration is decreased in the acute HF phase, and is altered during recovery.[33] The same study also reported that sNEP could be an indicator of hemodynamic alterations rather than HF severity. Thus, these changes in sNEP may enhance its effectiveness as an indicator of prognosis in HCM patients. Although we could not fully explain the reason why sNEP was not associated with prognosis in the present study, taking changes in sNEP into consideration, rather than 1-point testing, might be important.

Strengths and Limitations

The present study has several strengths. For instance, this is the first study to show the comprehensive associations of sNEP with natriuretic peptides, cardiac troponins, echocardiographic and hemodynamic parameters, and not with prognosis in HCM patients. Furthermore, no patients dropped out of the study.

The current study also has several limitations. First, as a cross-sectional and prospective cohort study of a single center with a relatively small number of patients, the study may be somewhat underpowered. HCM, however, is a relatively rare disease and thus the small number of subjects was unavoidable. Second, we did not perform genotyping in all subjects, and did not consider the differences between the various types of HCM. Third, the variables were measured only once on an outpatient basis or on hospitalization, without taking into consideration changes in medical parameters (e.g., sNEP). Fourth, although we encouraged catheterization as much as possible, we were not able to perform these measurements in all patients for various reasons (e.g., rejection by the patients, medical reasons). Thus, there may be potential selection bias in these measurements. And fifth, we measured BNP and NT-proBNP, but not other peptides such as atrial natriuretic peptide, bradykinin, substance P, adrenomedulin, endothelin-1 or angiotensin II. Therefore, the present results should be viewed as preliminary, and further studies with a larger population are needed.

Conclusions

Soluble neprilysin appears to correlate with BNP and cardiac systolic function, but it is not significantly associated with prognosis in HCM patients.

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The authors take responsibility for all aspects of the reliability and freedom from bias of the data presented, and for the discussed interpretation.

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Supplementary Files

Please find supplementary file(s);
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