ORIGINAL RESEARCH ARTICLE

Soluble neprilysin does not correlate with prognosis in pulmonary hypertension

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Abstract

Aims It has been reported that circulating soluble neprilysin (sNEP), which catalyses the degradation of several vasodilator peptides such as natriuretic peptides, predicts prognosis in heart failure patients with reduced ejection fraction. Hypoxia-induced decrease in NEP expression in lungs has been reported. However, the associations between sNEP and haemodynamic parameters, as well as the prognostic impact of sNEP in pulmonary hypertension (PH), remain unclear. We aimed to clarify the relationships between sNEP and natriuretic peptide, haemodynamics (e.g. parameters of echocardiography and right heart catheter) or prognosis in PH patients.
Methods and results First, we examined the associations between sNEP levels and natriuretic peptide, echocardiography, or right heart catheter in PH patients (mean pulmonary artery pressure $\geq$ 25 mmHg and pulmonary artery wedge pressure- 15 mm Hg on the basis of right heart catheterization, n = 79). Next, we followed up the patients for all-cause mortality. Laboratory data revealed no significant correlations between sNEP and B-type natriuretic peptide (R = 0.022, P = 0.872), N-terminal proBNP (R = −0.018, P = 0.872), and high-sensitivity troponin I (R = 0.206, P = 0.107). Regarding the parameters of echocardiography and right heart catheter, there were no significant correlations between sNEP and left ventricular ejection fraction (R = −0.036, P = 0.764), right ventricular fractional area change (R = −0.259, P = 0.064), tricuspid valve pressure gradient (R = −0.037, P = 0.767), and any of the right heart catheter parameters. In the Kaplan–Meier analysis (mean follow-up, 1284 days, log-rank P = 0.531), all-cause mortality rates were comparable between the higher NEP group (sNEP $\geq$ median levels of 1.45 ng/mL, n = 39) and the lower NEP group (sNEP < 1.45 ng/mL, n = 40). In the Cox proportional hazard analysis, sNEP was not a predictor of all-cause mortality (hazard ratio 0.902, 95% CI 0.674–1.207, P = 0.487) in PH patients.
Conclusions Circulating sNEP does not correlate with natriuretic peptide, haemodynamic parameters, or prognosis in patients with PH.

Introduction

Neprilysin (NEP) has been focused on since the recent publication of PARADIGM-HF trial in patients with heart failure (HF).[1,2] NEP is a membrane-bound enzyme that breaks down numerous vasoactive peptides and is widely expressed in the kidney, lungs, endothelial cells, vascular smooth muscle cells, cardiac myocytes, fibroblasts, neutrophils, adipocytes, testes, and brain, with the highest concentrations being present in the renal proximal tubules.[3–5] Furthermore, NEP catalyses the degradation of several vasodilator peptides, including natriuretic peptides, angiotensin II, bradykinin, substance P, adrenomedullin, and endothelin-1.[5] In a previous study, circulating NEP was detected in the sera of patients with HF.[6] In the same study, circulating levels of soluble NEP (sNEP) and its

activity showed a modest correlation, and circulating sNEP is biologically active in HF patients.[6] It has been reported that circulating sNEP predicts prognosis in HF patients with reduced ejection fraction (HFrEF) or acute decompensated HF patients,[4,5,7,8] whereas sNEP is not associated with prognosis in HF patients with preserved ejection fraction (HFpEF).[9] In the current study, it has been speculated that an HFpEF primarily arose from right ventricular dysfunction and pulmonary vascular disease, that NEP may exert differential effects in pulmonary vs. systemic circulation, and that the prognostic impact of NEP in HFpEF differs from that of HFrEF.[9]

Pulmonary hypertension (PH) is characterized by elevated pulmonary arterial pressure due to vasoconstriction and remodelling of the pulmonary microvasculature, which leads to right ventricular failure and death.[10] In the pulmonary circulation, protective effect of NEP has also been reported, by attenuating the growth of vascular smooth muscle cells.[11] Increased NEP activity and/or expression following exposure to hypoxia has been reported.[11–14] Furthermore, hypoxia-induced decrease in NEP expression in lungs has been reported.[15] The associations between sNEP and haemodynamic parameters, as well as its prognostic impact in PH, have never been reported. Although several biomarkers such as uric acid, bilirubin, creatinine, C-reactive protein, natriuretic peptides,[16,17] and cardiac troponins[18] have been reported as prognostic biomarkers, there is no fully established biomarker in PH patients.[10,19] Therefore, we examined whether sNEP will be a novel biomarker of PH.

We aimed to clarify the relationships between sNEP and natriuretic peptide, haemodynamics (e.g. parameters of echocardiography and right heart catheter), or prognosis in PH patients.

Methods

Subjects and study protocol

This is a prospective observational study that enrolled consecutive pre-capillary PH patients [pulmonary artery pressure (PAP) ≥ 25 mmHg and pulmonary artery wedge pressure (PAWP) ≤ 15 mm Hg based on right heart catheterization (RHC)][10] who had been admitted to Fukushima Medical University Hospital for diagnosis and treatment between 2009 and 2016. These patients (mean PAP 42.9 ± 14.5 mmHg, $n = 79$) were classified into the following groups: pulmonary arterial hypertension (Group 1, 41 patients); PH due to lung disease (Group 3, four patients); chronic thromboembolic PH (Group 4, 30 patients); and others (Group 5, four patients).[10] There were no patients who had previously taken NEP inhibitors or undergone pulmonary endarterectomy and/or lung transplantation. All RHCs were performed with

the patients in a stable condition as previously reported.[20] Echocardiography was performed by experienced echocardiographers using standard techniques within 3 days of RHC as previously reported.[21] After overnight fasting, blood sample was obtained from each patient within 3 days of RHC, regardless of presence or absence of medications for PH, and the circulating levels of plasma sNEP was measured by radioimmunoassay (ELH-Neprilysin-1 kit, RayBiotech, Inc, Norcross, GA, USA). These patients were finally divided into two groups on the basis of their median sNEP levels: low (sNEP < 1.45 ng/mL, $n = 40$) and high groups (sNEP ≥ 1.45, $n = 39$).

Firstly, we compared the clinical features and results from RHC, laboratory tests, and echocardiography between the two groups. In addition, we performed a correlation analysis of interaction between levels of sNEP and parameters of laboratory data, echocardiography, and RHC. Secondly, the patients were followed up until 2018 for all-cause death. We were able to follow up all patients. Status and dates of death were obtained from the patients' medical records. If these data were unavailable, status was ascertained by a telephone call to the patient's referring hospital physician. Those administering the survey were blind to the analyses, and written informed consent was obtained from all study 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.[22]

Statistical analysis

Normally distributed data are presented as mean ± SD, and non-normally distributed data are presented as median and inter-quartile range. The categorical variables are expressed as numbers and percentages, and the χ^2 test was used for their comparisons. 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 the parameters of laboratory data, echocardiography, and RHC were assessed using Pearson's correlation analysis for parametric variables and Spearman's correlation analysis for non-parametric variables. The Kaplan–Meier analysis was used with a log-rank test to assess mortality. The Kaplan–Meier estimates of the survival curves for the two groups were plotted against the time-to-follow-up period. Cox proportional hazard analyses were used to evaluate sNEP (categorical variable) and high sNEP (continuous variable) levels as a predictor of all-cause mortality. A value of $P < 0.05$ was considered statistically significant for all comparisons. These analyses were performed

using a statistical software package (SPSS ver. 24.0, IBM, Armonk, NY, USA).

Results

The clinical features of the present study's subjects are summarized in *Table 1*. Any clinical features did not significantly differ between the high and low sNEP groups. Regarding the parameters of laboratory data, echocardiography or RHC did not differ between the two groups. There was no significant correlation between sNEP and any of these parameters.

During the follow-up period (mean 1284 ± 770 days, range 48–2873 days), 13 patients died owing to complications related to PH (sudden death, heart failure, and/or respiratory failure). As shown in *Figure 1*, in the Kaplan–Meier analysis, the all-cause mortality rates were comparable between the two groups (log-rank *P* = 0.531). In the Cox proportional hazard analysis, neither sNEP (as continuous variable, *P* = 0.487)

Table 1 Comparisons of background characteristics (*n* = 79)

	Low group (sNEP < 1.45, *n* = 40)	High group (sNEP ≥ 1.45, *n* = 39)	*P*-value	Correlation to sNEP, *R*	*P*-value
Neprilysin (ng/mL)[a]	0.76 (0.51–0.93)	2.32 (1.84–4.01)	<0.001		
Demographics					
Age (years)	58.2 ± 17.1	54.2 ± 17.7	0.301	−0.131	0.249
Male gender (*n*, %)	14 (35.0)	8 (20.5)	0.151		
Body mass index (kg/m^2)	22.9 ± 4.1	22.8 ± 4.6	0.957	−0.025	0.829
Systolic BP (mmHg)	120.6 ± 20.3	116.3 ± 16.3	0.312	−0.027	0.812
Diastolic BP (mmHg)	70.0 ± 11.2	68.7 ± 11.7	0.606	0.050	0.659
Heart rate (b.p.m.)	81.1 ± 18.0	80.3 ± 18.0	0.848	0.034	0.766
WHO functional class I/II/III/IV	4/30/6/0	5/31/3/0	0.573		
PH classification group 1/3/4/5	16/3/19/2	25/1/11/2	0.165		
Right heart catheterization					
PAP mean (mmHg)	43.9 ± 14.3	42.0 ± 14.8	0.581	−0.043	0.718
PAP systolic (mmHg)	71.2 ± 20.5	67.0 ± 22.3	0.409	−0.076	0.521
PAP diastolic (mmHg)	28.5 ± 13.0	28.5 ± 17.1	0.997	0.012	0.920
RA pressure mean (mmHg)	6.5 ± 4.4	7.7 ± 5.0	0.272	0.178	0.137
PAWP mean (mmHg)	11.2 ± 6.1	10.5 ± 5.2	0.565	−0.057	0.642
Cardiac output (L/min)	4.1 ± 1.0	4.2 ± 1.5	0.652	0.025	0.839
Cardiac index (L/min/m^2)	2.6 ± 0.7	2.8 ± 0.9	0.412	0.045	0.709
PVR (dyne·s/cm^5)	655.9 ± 339.0	665.8 ± 43.5	0.918	−0.004	0.976
Laboratory data					
N-terminal proBNP (pg/mL)[a]	577.8 (150.7–1482.0)	746.4 (119.7–1371.0)	0.268	−0.018	0.872
BNP (pg/mL)[a]	113.0 (26.8–203.6)	110.0 (33.6–278.3)	0.646	0.022	0.860
Total bilirubin (mg/dL)	1.3 ± 0.9	1.2 ± 0.8	0.790	0.063	0.596
Creatinine (mg/dL)	0.8 ± 0.3	0.7 ± 0.2	0.195	−0.100	0.403
Sodium (mEq/L)	139.9 ± 2.7	139.7 ± 3.7	0.716	0.033	0.782
Uric acid (mg/dL)	6.0 ± 1.9	6.4 ± 2.2	0.359	0.198	0.102
C-reactive protein (mg/dL)[a]	0.12 (0.06–0.53)	0.18 (0.06–0.50)	0.739	0.083	0.489
Troponin I (ng/mL)[a]	0.008 (0.005–0.016)	0.014 (0.006–0.021)	0.507	0.206	0.107
Echocardiography					
LVEF (%)	63.8 ± 9.3	61.7 ± 14.0	0.460	−0.036	0.764
Left atrial volume (mL)	48.8 ± 53.5	45.2 ± 32.8	0.746	0.012	0.927
Mitral valve E/e′	10.7 ± 4.4	11.4 ± 6.6	0.620	0.013	0.921
RA diameter long (mm)	50.9 ± 10.1	51.8 ± 13.5	0.780	0.056	0.689
RA diameter short (mm)	37.8 ± 10.6	39.9 ± 8.4	0.441	0.163	0.249
RA end-systolic area (cm^2)	19.4 ± 8.1	20.5 ± 9.8	0.674	0.107	0.446
RV diameter base (mm)	40.2 ± 8.5	38.8 ± 7.8	0.539	0.019	0.779
RV diameter mid (mm)	32.9 ± 10.1	32.9 ± 8.8	0.978	0.086	0.537
RV diameter long (mm)	73.2 ± 9.7	72.2 ± 9.9	0.709	−0.021	0.881
RV area diastole (cm^2)	22.4 ± 9.3	22.1 ± 8.0	0.907	0.067	0.632
RV area systole (cm^2)	15.1 ± 8.0	15.2 ± 5.6	0.948	0.185	0.189
RV-FAC (%)	34.8 ± 10.8	31.1 ± 14.7	0.304	−0.259	0.064
IVC (mm)	14.6 ± 4.8	15.6 ± 4.7	0.382	0.072	0.550
TRPG (mmHg)	58.5 ± 31.8	51.3 ± 26.8	0.312	−0.037	0.767
Tricuspid valve S′ (cm)	8.3 ± 2.7	9.3 ± 5.0	0.445	0.064	0.713
Tricuspid valve E/e′	5.8 ± 3.7	5.1 ± 4.0	0.614	−0.125	0.468

BNP, B-type natriuretic peptide; BP, blood pressure; IVC, inferior vena cava diameter; LVEF, left ventricular ejection fraction; mitral valve E/e′, ratio of the peak transmitral velocity during early diastole to the peak mitral valve annular velocity during early diastole; sNEP, soluble neprilysin; PAP, pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; RA, right atrial; RV, right ventricle; RV-FAC, right ventricular fractional area change; TRPG, tricuspid regurgitation pressure gradient; WHO, World Health Organization.
[a]Presented as median (inter-quartile range).

Figure 1 Kaplan–Meier analysis for all-cause mortality stratified by soluble neprilysin (sNEP) levels.
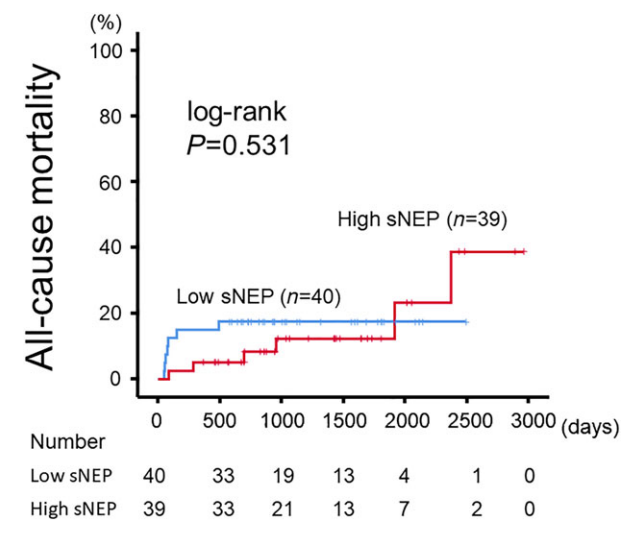
nor high sNEP (as categorical variable, $P = 0.533$) levels were a predictor of all-cause mortality in PH patients, including each type of PH classification (data not shown).

Discussion

The associations between sNEP and haemodynamic parameters, as well as its prognostic impact in PH, have never been reported. To the best of our knowledge, the present study is the first to report that sNEP does not correlate with any parameters of RHC, echocardiography, or laboratory data, including natriuretic peptides and troponin I, and is not associated with prognosis in PH patients.

To date, the mechanism of action of NEP remains poorly understood in patients not only with PH but also with HF.[3,23] NEP substrates having peripheral vasodilation include natriuretic peptides, bradykinin, substance P, and adrenomedullin, and those with peripheral vasoconstriction include angiotensin II and endothelin-1.[3] Net effects of NEP on vascular tone will depend on whether the predominant substrates degraded are vasodilators or vasoconstrictors,[3] and this balance may contribute to promoting PH. Thus, it seems reasonable that we found no relationship between sNEP levels and RHC parameters. In addition, no relationship has been found between sNEP concentration and natriuretic peptides levels in HF patients.[4–8] Concordant with these findings,[4–8] we did not find associations between sNEP and natriuretic peptide levels in the current PH patients. Inhibiting NEP will augment naturally occurring natriuretic peptides, which promote natriuresis, induce vasodilation, and reduce cardiac hypertrophy and fibrosis in HF patients.[5] To my knowledge, there are no previous reports on associations between sNEP and haemodynamics nor on impact of inhibiting NEP in PH patients.

With regard to sNEP and prognosis in HF patients, Byes-Genis *et al*. reported that circulating higher NEP is associated with cardiovascular prognosis, particularly cardiovascular mortality and HF hospitalization, independently from natriuretic peptides.[5] Conversely, in a recent observational registry of 144 patients with HFpEF, Goliasch *et al*. could not confirm an association between NEP levels and cardiovascular mortality or hospitalization for HF, in contrast to HFrEF.[9] The sNEP levels in the HFpEF patients were three-fold higher than in the HFrEF patients.[9] This mismatch between sNEP and its target protein levels might explain the lack of correlation between NEP levels and prognosis/functional measures in HFpEF.[9] In the current study, no relationships between sNEP and parameters of echocardiography or RHC were observed, which was concordant with a previous report of patients with HFpEF.[9] In the said study, there were no significant correlations between sNEP levels and left ventricular filling pressures or fibrosis as assessed by cardiac magnetic resonance imaging or myocardial biopsy.[9] In addition, changes in sNEP in HF with haemodynamics are controversial,[24,25] Takahama *et al*. recently reported that sNEP concentrations did not change from admission to before discharge.[25] Conversely, Arrigo *et al*. recently reported that sNEP concentrations are impaired in phase of acute heart failure and altered during recovery from acute heart failure, and sNEP could be an indicator of haemodynamic alterations rather than HF severity.[24] Thus, these changes in sNEP during follow-up period, which were not estimated in the present study, are possible to affect insufficient predictor of sNEP on PH patients.

In the lung, membrane-bound NEP appears within airways in cells that are associated with tachykinin receptors.[23] NEP is present in the basal cells of airway epithelium, nervous, smooth muscle, glands, and blood vessels.[23] Hypoxia-induced decrease of NEP expression in lung, but not in serum, has been reported.[15] In the present study, we could not deny differences in the effect of NEP expression in lung and sNEP levels on the haemodynamic parameters and prognosis. NEP may protect the lung against hypoxia-induced vascular remodelling, in large part by limiting the magnitude of neuropeptide-induced proliferative, migratory, and/or contractile responses.[11] The production of soluble/non-membrane-associated counterparts of membrane-bound proteins has been studied extensively and is known to occur as a consequence of ectodomain shedding, which involves the proteolytic cleavage of the extracellular domain, or release of non-membrane-associated enzymes from cells via exosomes. With respect to PH, sildenafil plus NEP inhibitor, ecadotril, which increases endogenous natriuretic peptide levels, decreases PAP and right ventricular hypertrophy.[26]

Study limitations

The current study has several limitations. First, as a prospective cohort study of a single centre with a relatively small

number of patients, the study may be somewhat underpowered. However, PH is a relatively rare disease, and our study population was not smaller than those of previous studies.[17,18] Second, we used only variables on hospitalization in this study, without taking into consideration changes in medical parameters (e.g. sNEP) or post-discharge treatment. Third, we have measured BNP and N-terminal proBNP but not other peptides including bradykinin, substance P, adrenomedullin, endothelin-1, and angiotensin II. Therefore, the present results should be viewed as preliminary, and further studies with a larger population are needed.

Conclusions

To our knowledge, the present study is the first to report that sNEP does not correlate with any parameters of RHC, echocardiography, or laboratory data, including natriuretic peptides and troponin, and is not associated with prognosis in PH patients.

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Conflict of interest

A.Y. and T.M. belong to the Department of Advanced Cardiac Therapeutics, supported by Fukuda-denshi Co, Ltd. This company is not associated with the contents of the current study. T.Y. and K.S. belong to the Department of Pulmonary Hypertension, supported by ACTELION PHRAMA Co, Ltd. This company is also not associated with the contents of the current study.

Author Contributions

A.Y. drafted the article and designed this study. T.Y. and T.M. performed statistical analysis. M.O., A.K., T.Y., K.S., H.K. and K.N. obtained general data. Y.T. designed this study, obtained general data, and revised the article critically for important intellectual content. All authors read and approved the final manuscript.

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