ORIGINAL ARTICLE

Soluble neprilysin and survival in critically ill patients

Max Lenz[1,2] (iD), Konstantin A. Krychtiuk[1,2]*, Mira Brekalo[1], Dominik F. Draxler[1], Noemi Pavo[1], Christian Hengstenberg[1], Kurt Huber[2,3], Martin Hülsmann[1], Gottfried Heinz[1], Johann Wojta[1,2,4] and Walter S. Speidl[1]

[1]Division of Cardiology, Department of Internal Medicine II, Medical University of Vienna, Spitalgasse 23, Vienna, 1090, Austria; [2]Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna, Austria; [3]3rd Medical Department, Wilhelminenhospital, Vienna, Austria; and [4]Core Facilities, Medical University of Vienna, Vienna, Austria

Abstract

Background Critically ill patients admitted to an intensive care unit (ICU) exhibit a high mortality rate irrespective of the initial cause of hospitalization. Neprilysin, a neutral endopeptidase degrading an array of vasoactive peptides became a drug target within the treatment of heart failure with reduced ejection fraction. The aim of this study was to analyse whether circulating levels of neprilysin at ICU admission are associated with 30 day mortality.

Methods and results In this single-centre prospective observational study, 222 consecutive patients admitted to a tertiary ICU at a university hospital were included. Blood was drawn at admission and soluble neprilysin levels were measured using ELISA. In the total cohort, soluble neprilysin levels did not differ according to survival status after 30 days as well as type of admission. However, in patients after surgery or heart valve intervention, 30 day survivors exhibited significantly lower circulating neprilysin levels as compared to those who died within 30 days (660.2, IQR: 156.4–2512.5 pg/mL vs. 6532.6, IQR: 1840.1–10 000.0 pg/mL; P = 0.02). Soluble neprilysin predicted mortality independently from age, gender, and commonly used scores of risk-prediction (EuroSCORE II, STS-score, and SAPS II score). Additionally, soluble neprilysin was markedly elevated in patients with sepsis and septic shock (P < 0.05).

Conclusion At the time of ICU admission, circulating levels of neprilysin independently predicted 30 day mortality in patients following cardiac surgery or heart valve intervention, but not in critically ill medical patients. Furthermore, patients suffering from sepsis and septic shock displayed significantly increased circulating neprilysin levels.

Introduction

Patients admitted to medical intensive care units (ICUs) display various underlying pathologies ranging from haemodynamic instability over infections to severe organ dysfunction. Despite the heterogeneity of mentioned conditions, critically ill patients often exhibit similarities in specific vital parameters or biomarkers throughout the ICU stay.

Neprilysin is a metalloprotease that cleaves a broad spectrum of peptides and inactivates several peptide hormones under physiologic conditions.[1] It has been isolated from various organs[2–3] and although a membrane-bound occurrence was assumed at first, a soluble form of neprilysin (sNEP) was discovered within the circulation.[4,5] The exact mechanism behind the release of sNEP remains only partly understood and might range from separate production of a non-membrane-associated form to ectodomain-shedding or transport via exosomes.[6] From a cardiovascular point of view, NEP plays a crucial role in modulating the activity of natriuretic peptides (NPs) by degrading them.[7] Its physiologic role and the occurrence of a soluble form of NEP constitutes it a promising biomarker.[8]

In a large cohort of patients suffering from heart failure (HF) with reduced ejection fraction, circulating levels of NEP were significantly associated with the composite endpoint of cardiovascular death and hospitalization due to heart failure.[9] Although their physiological effects seem to be

intertwined, sNEP and NT-proBNP did not show a significant correlation. Interestingly, sNEP was substantially less influenced by comorbidities and remained an independent prognostic factor in an applied multimarker strategy as compared with NT-proBNP.[10] Although NEP was examined as a biomarker in different cohorts including HF and non-cardiac collectives, data on its role in critically ill patients are scarce, while due to its physiologic properties, it is promising. We therefore aimed to illuminate the prognostic properties of NEP in an unselected cohort of ICU patients admitted to a medical ICU.

Materials and methods

Subjects and study design

For this single-centre prospective observational study, we included all consecutive patients admitted to the medical ICU of the Department of Internal Medicine II (Medical University of Vienna) between August 2012 and August 2013. Patients under the age of 18 years, as well as all patients with active HIV or HCV infection, were not included to minimize the risk of transmission. The study was approved by the local ethics committee of the Medical University of Vienna (EK 1101/2012) and complies with the Declaration of Helsinki. Conscious patients had to give informed consent, while for unconscious patients, the need for informed consent was waived by the ethics committee. Our centre is a tertiary care medical ICU, assigned to treat the entire spectrum of critical illness with a focus on acute cardiovascular diseases. In addition to medical patients, we are provided with patients undergoing major heart and thoracic surgery as well as patients after catheter-based valve interventions. At the time of admission, baseline demographics, the primary cause of admission, clinical history, laboratory values, and vital parameters were recorded. Additionally, major interventions preceding ICU admission or taking place within the first 72 h of the ICU stay were recorded and included; mechanical ventilation, major surgery, extracorporeal renal replacement therapy, extracorporeal membrane oxygenation, and the use of catecholamines. The simplified acute physiology score II (SAPS II) and the sequential organ failure assessment score (SOFA) were used to assess the severity of disease.[11–13]

Additionally, the EuroSCORE II as well as the STS score (Society of Thoracic Surgeons Score) were calculated to evaluate procedural risk.[14–15] Data regarding 30 day mortality was collected. A total of 233 patients were included; for 222 patients sNEP levels were available. All further analyses were conducted within the available patients. No patients were lost to follow-up.

Blood sampling

Blood was drawn at the time of admission (24 h time window) from an arterial or central venous line. A serum separator tube, an EDTA-tube and a 3.8% sodium citrate vacuette tube (Greiner Bio-One, Austria) were used for collection after discarding the initial 3 mL of blood to ensure stable sampling conditions. Consecutively, samples were centrifuged at 4°C and 3000 RPM for 15 min and stored at −80°C for later analysis. Standard laboratory values including NT-proBNP were carried out by the department of laboratory medicine of the Vienna general hospital.

Soluble neprilysin measurement

Soluble neprilysin was measured using commercially available ELISA-kits (Human Neprilysin DuoSet ELISA, R&D Systems, USA) according to the manufacturer's instructions. Optical density was determined using a microplate reader set to 450 nm.

Statistical analysis

Sample size calculation revealed that in a cohort with an estimated mortality rate of 25%, given a power of 0.8 and a significance level of 0.05, 218 patients would be required to detect a difference of 50% in sNEP levels between survivors and non-survivors. Categorical variables are summarized as counts and percentages. They are compared by the χ^2 or by Fisher's exact test as appropriate. Continuous variables (determined by the Kolmogorov–Smirnov test) are displayed as median and interquartile range (IQR). Data were compared by the Mann–Whitney U test; multiple groups were compared by Kruskal–Wallis one-way analysis of variance. Kaplan–Meier analysis (log-rank test) was applied to verify the time-dependent discriminative power of sNEP values above and below the median of the observed population. Cox proportional hazard regression analysis was used to assess the independent prognostic value of sNEP on mortality. Two-sided P values of <0.05 were considered statistically significant. SPSS 26.0 (IBM Corporation, USA) was used for all statistical analyses.

Results

Baseline characteristics

The demographic and clinical characteristics of the 222 included patients at baseline are given in *Table 1*. Seventy-one patients underwent cardiac surgery or heart valve intervention (*Table 2*), while 151 patients were

Table 1 Clinical and demographic baseline characteristics of the study population

	Total (*n* = 222)	Medical (*n* = 151)	Cardiac surgery and valve intervention (*n* = 71)	*P* value
Age (years)	67.0 (54.9–76.7)	65 (52.7–75.9)	69.6 (59.2–77.5)	0.14
Male gender, *n* (%)	133 (59.9%)	100 (66.2%)	37 (52.1%)	0.53
Vasopressor use, *n* (%)	130 (58.6%)	85 (56.3%)	45 (63.4%)	<0.001
Mechanical ventilation, *n* (%)	130 (58.6%)	89 (58.9%)	41 (57.7%)	0.013
ECMO, *n* (%)	13 (5.9%)	9 (6%)	4 (5.6%)	1.00
Creatinine (mg/dL)	1.2 (0.9–2.0)	1.1 (0.9–1.7)	1.7 (1.2–2.8)	<0.001
Bilirubin (mg/dL)	0.9 (0.5–1.5)	0.8 (0.5–1.3)	1.1 (0.6–1.8)	0.03
Lactate (mmol/L)	1.9 (1.2–3.2)	1.7 (1.2–2.7)	2.8 (1.3–6.7)	0.002
C-reactive protein (mg/dL)	3.9 (1.2–10.7)	3.5 (0.9–10.6)	4.8 (2.2–10.9)	0.14
Procalcitonin (ng/mL)	0.4 (0.1–1.8)	0.3 (0.1–1.0)	1.3 (0.4–5.0)	<0.001
Leucocytes (G/L)	9.2 (7.0–13.5)	8.9 (7.1–13.4)	10.3 (6.5–15)	0.35
SAPS II score	44 (31–57)	49 (38–63)	32 (27–42)	<0.001
STS score	—	—	2.56 (1.42–6.05) vs. 3.63 (2.50–5.64)	0.164
EuroSCORE II	—	—	2.82 (1.43–6.21) vs. 5.26 (2.04–10.62)	0.140

ECMO, extracorporeal membrane oxygenation; SAPSII, Simplified Acute Physiology Score; STS score, Society of Thoracic Surgeons Score. Numbers are given as total count (*n*) and percentages (%) or as median and interquartile range. STS score is given for survivors vs. non-survivors as median and interquartile range.

Table 2 Type of valve intervention and cardiac surgery (*n* = 71)

Heart valve intervention	N = 26 (%)
TAVI	21 (80.8%)
Edge-to-edge mitral valve repair	5 (19.2%)
Cardiac surgery	N = 45 (%)
Acute surgery	11 (24.44%)
CABG	15 (33.33%)
Valve surgery	11 (24.44%)
CABG + valve surgery	10 (22.22%)
Other	9 (20.0%)

CABG, coronary artery bypass grafting; TAVI, transcatheter aortic valve implantation. Numbers are given as total count (*n*) and percentages (%).

Table 3 Primary diagnosis of medical patients admitted to the intensive care unit (*n* = 151)

| Primary diagnosis:	N = 151 (100%)
Cardiopulmonary resuscitation	49 (32.45%)
Heart failure or cardiogenic shock	48 (31.79%)
Sepsis	19 (12.58%)
Pulmonary disease	16 (10.60%)
Other	19 (12.58%)

Numbers are given as total count (*n*) and percentages (%).

admitted for medical reasons (*Table 3*). The 30 day mortality of medical patients was 35.1%; surgical patients and patients after heart valve intervention displayed a mortality rate of 7.1%.

Soluble neprilysin levels at admission and primary diagnosis

The sNEP levels at admission did not differ between medical and surgical patients (551.1, IQR: 237.1–3185.0 pg/mL vs. 972.6, IQR: 234.4–3012.3 pg/mL; *P* = 0.75; *Figure 1A*). There was also no difference in sNEP levels in patients after cardiac surgery (1040.8, IQR: 312.3–2689.6 pg/mL; *n* = 45) and heart valve intervention (785.8, IQR: 71.7–7520.8; *n* = 26; *P* = 0.63; *Figure 1B*). *Figure 1C* shows sNEP levels of medical patients according cause of ICU admission. Interestingly, patients with acute heart failure and cardiogenic shock did not show elevated sNEP levels as compared with medical patients without heart failure. In contrast, sNEP was markedly elevated in patients with sepsis and septic shock (*P* < 0.05). sNEP did not correlate with the cardiac markers NT-proBNP (*R* = 0.06; *P* = 0.42) and troponin T (*R* = −0.04; P = 0.53). Medical

patients displayed significantly higher NT-proBNP levels (4402 pg/mL, IQR: 1370.5–15765.0 pg/ml vs. 2032 pg/mL, IQR: 818.0–4415.0 pg/mL; P < 0.001, data not shown) as compared with surgical/interventional patients. Patients requiring extracorporeal membrane oxygenation (ECMO) displayed significantly higher levels of sNEP compared with patients without ECMO (1818.6, IQR: 701.8–10 000 pg/mL vs. 551.1, IQR: 206.6–2893.3 pg/mL, *P* = 0.02). In addition, sNEP was not associated with renal function (serum creatinine: *R* = 0.12; *P* = 0.08; blood urea nitrogen: *R* = 0.05; *P* = 0.47) or the inflammatory parameters C-reactive protein (*R* = −0.02; *P* = 0.77), procalcitonin (*R* = 0.11; *P* = 0.11) or leucocyte count (*R* = −0.04; *P* = 0.56). In contrast, sNEP correlated with serum lactate (*R* = 0.20; *P* = 0.004) at ICU admission.

Neprilysin predicts mortality in surgical but not in medical patients

In the total cohort, sNEP levels did not differ between 30 day survivors and non-survivors (669.5, IQR: 235.8–3098.7 pg/mL vs. 599.0, IQR: 252.8–2919.9 pg/mL; *P* = 0.83; *Figure 2A*). However, in surgical patients and patients after catheter-based valve interventions, sNEP levels were significantly lower in survivors than non-survivors (660.2, IQR:

Figure 1 Neprilysin levels at admission to the intensive care unit. Soluble neprilysin levels of medical vs. surgical and valve intervention patients, at time of admission to the intensive care unit (A); levels of neprilysin in patients after cardiac surgery or heart valve intervention (B); neprilysin levels in medical patients according to primary diagnosis (C); *$P < 0.05$.
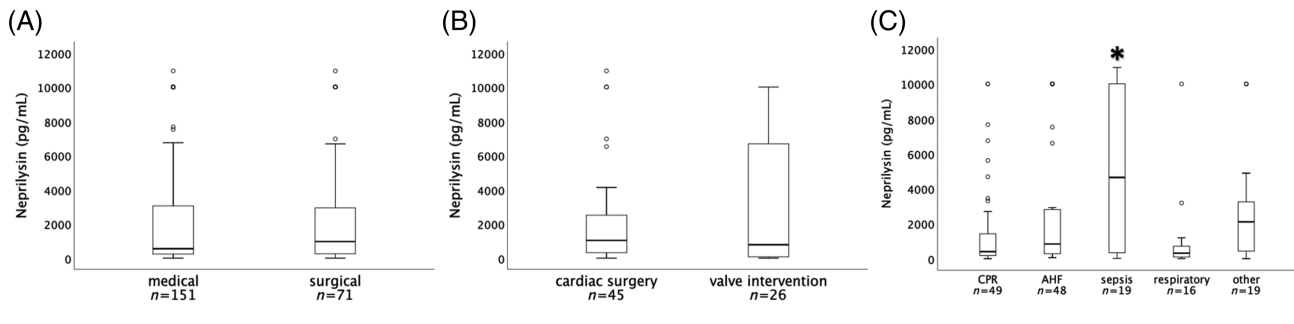
Figure 2 Neprilysin levels and 30 day survival. Serum levels of neprilysin in 30 day survivors and non-survivors in the total cohort (A), in patients that were admitted due to cardiac surgery or heart valve intervention (B) and medical patients (C); *$P < 0.05$.
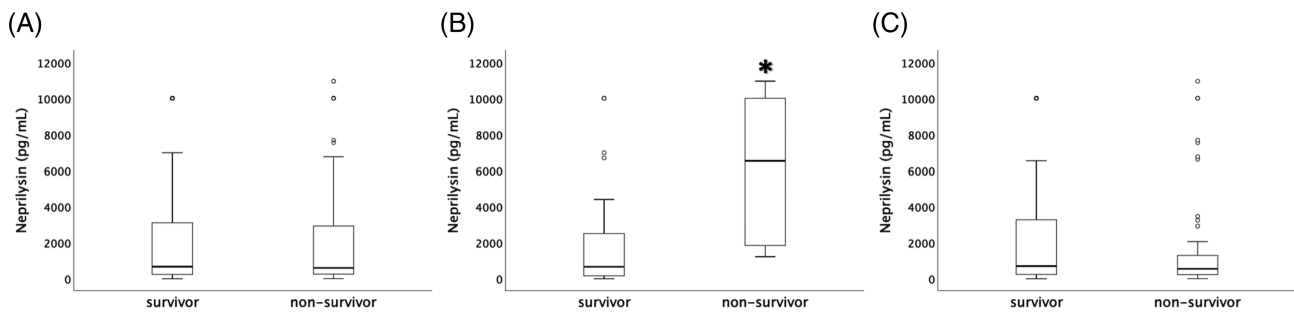
Figure 3 Survival according to neprilysin levels above or below the median. Survival according to neprilysin levels above (red line) or below (blue line) the median in the total cohort (A), in patients that were admitted due to cardiac surgery or heart valve intervention (B) and in medical patients (C).
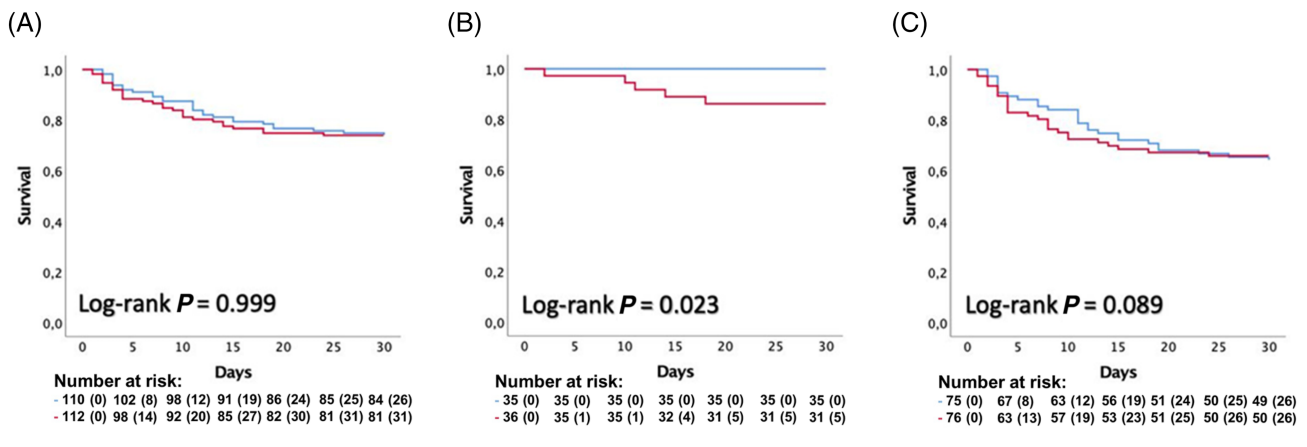
156.4–2512.5 pg/ml vs. 6532.6, IQR: 1840.1–10000.0 pg/mL; $P = 0.02$; *Figure 2B*). Conversely, sNEP was not associated with survival in medical patients (survivors: 698.9, IQR: 237.1–3263.3 pg/mL; non-survivors: 548.5, IQR: 224.1–1290.7 pg/mL; $P = 0.32$; *Figure 2C*). sNEP levels above the median were significantly associated with mortality in patients after cardiac surgery or valve intervention ($P < 0.05$) but not in medical patients (*Figure 3*). Cox-regression revealed that sNEP predicted mortality independent from age, gender, and the EuroSCORE II (hazard ratio for one standard

deviation increase of neprilysin: 2.65, 95% confidence interval 1.04–6.753; *P* = 0.041). Similar results were found using sNEP, age, gender, and the STS-score (hazard ratio for one standard deviation increase of neprilysin: 4.52, 95% confidence interval 1.379–14.821; *P* = 0.013) or the SAPS II score (hazard ratio for one standard deviation increase of neprilysin: 2.35, 95% confidence interval 1.02–5.41; *P* = 0.044).

Discussion

Within this single-centre, prospective observational study including 222 critically ill patients we were able to elucidate the prognostic value of sNEP in an unselected cohort of patients admitted to a tertiary intensive care unit. Within the total population, sNEP levels at admission displayed no prognostic merit regarding 30 day mortality. Considering the heterogeneous causes of admission, patients were assigned into two groups for further analyses: medical patients and patients undergoing cardiac surgery or heart valve intervention. At the time of admission, sNEP levels did not differ between the two described groups. However, patients undergoing cardiac surgery or heart valve intervention showed significantly increased circulating levels in 30 day-non-survivors. Furthermore, sNEP-levels above the median of the observed population were significantly associated with mortality. Additionally, sNEP was found to be a predictor of mortality independent of age, gender, and commonly used scores of risk prediction (EuroSCORE II, STS-score, and SAPS II score).

In the medical cohort of this study, 30 day mortality was independent of circulating sNEP-levels. This might be due to the heterogeneity of the observed population and the varying stages of critical illness. For individual pathologies, the use of single natriuretic peptides as risk predictors seems to be more feasible. NT-proBNP was proven to exert diagnostic and prognostic value in cardiac dysfunction and hypoxic respiratory failure.[16] Pro-ANP was found to be a valuable prognostic biomarker in severe sepsis and septic shock.[17] In critically ill patients, NT-proCNP was associated with inflammatory parameters and markers of organ dysfunction.[18] Although NEP is ramified in the clearance of all natriuretic peptides, their individual pathophysiological involvement could hinder NEPs merged prognostic merit. Furthermore, the biological activity of sNEP as compared to the membrane-bound form of NEP is still a topic of discussion. Whether elevated levels of sNEP are caused by increased demand of substrate-cleaving or by release during cellular stress and/or damage remains unclear.

Patients undergoing cardiac surgery or catheter-based valve intervention displayed significantly increased sNEP levels in 30 day-non-survivors. As described previously, NEP was reported to cleave a wide variety of substrates. Thus, a

key role in binding and clearing natriuretic peptides was already established for the membrane-bound form.[19] A recent study underlined the connection of cardiac endocrine response and NEP in patients suffering from HF. After the implantation of a total artificial heart, MR-proANP, BNP, and sNEP levels were found drastically reduced.[20] This might hint towards a predominant cardiac regulation of sNEP. Given the prior reports on the prognostic value of natriuretic peptides for the survival of patients undergoing cardiac surgery and catheter-based valve interventions, our data might align with these findings, as the majority of the patients in this group underwent valve and or bypass surgery. For aortic stenosis, a link to elevated NEP content as well as NEP enzymatic activity was already established in cardiomyocytes.[21] Elevated peak postoperative BNP levels predicted long-term physical function after primary CABG surgery.[22] Again, as most of the discussed findings are applicable for membrane-bound NEP, the influence of NPs on sNEP remains likely, but unproven. Within a population undergoing major surgery/interventions such as ours, the presence of significant cellular stress as well as tissue damage and consequent sNEP release seems likely. Following these assumptions, sNEP could be indirectly affected by factors such as cardiac wall stress, haemodynamic aspects, temporary ischemia, tissue damage, and inflammatory involvement, circumstances as they occur following cardiac surgery and catheter-based valve interventions with worse outcome. Moreover, the procedures themselves may directly cause the rise in sNEP. The described prognostic properties within the surgical/interventional collective may be caused by the homogeneous underlying pathologies. Among the non-survivors in this study, a high percentage (80%) of patients required postoperative ECMO implantation and consecutively died from multiple organ failure. Whether these patients had initially high sNEP levels before surgery due to advanced cardiac disease, which leads to a complicated periprocedural course requiring ECMO support or whether periprocedural factors lead to an increase of sNEP after surgery cannot be answered by our study. However, a combined causality seems plausible.

When comparing medical patients to those undergoing cardiac surgery/heart valve intervention, several findings warrant further discussion. Although medical patients displayed significantly higher NT-proBNP levels, sNEP did not differ between medical and surgical/interventional patients. This might hint towards a release during cellular stress and/or damage rather than a regulation by NPs. However, among NPs, BNP was reported to be relatively resilient to the degradation exerted by NEP. Furthermore, medical patients showed significantly higher SAPS II scores on average. It was previously described that BNP might even act as an endogenous neprilysin inhibitor, marking low NEP levels in patients suffering from severe HF.[23] This might be partially applicable to our medical cohort comprised of critically ill patients with a variety of underlying heart diseases.

In the total cohort, NEP was found to be associated with serum lactate at the time of admission. Lactate levels are crucially influenced by the general haemodynamic status and oxygen supply. Given the previously discussed presumption of holistically influenced sNEP levels, this finding might be explained. Interestingly, when comparing the primary diagnosis leading to ICU admission, patients suffering from sepsis and septic shock displayed significantly increased levels of sNEP. Previous studies already demonstrated the occurrence of a membrane-bound NEP-form (CD10) in human neutrophils. The percentage of NEP (CD10) positive neutrophils was found to be linked with the severity of infection and the clinical outcome of the observed patients.[24] In addition to reporting a decreased NEP (CD10) expression in patients with septic shock, the work of Martens *et al*. proposes a decrease throughout the time of infection.[25] An additional link between NEP and sepsis can be found in the regulation of adrenomedullin (ADM). ADM regulates endothelial permeability as well as vascular tone during sepsis. Within the AdrenOSS-1 study associations between bio-ADM upon admission to the ICU and 28 day mortality as well as the SOFA score were observed.[26] Recently published, Arfsten *et al*. connected ARNi-treatment to a significant increase of MR-proADM and bio-ADM in patients suffering from heart failure with reduced ejection fraction, indirectly confirming an association of NEP and ADM.[27] Connecting these findings with our data, one could assume an association of decreasing membrane-bound NEP on neutrophils and increased levels of sNEP. Whether this is caused by ectodomain shedding, or cell death remains speculative. Due to the nature of our study design, membrane-bound NEP was not measured, leaving a clear answer to the balance of membrane-bound and soluble NEP beyond the scope of this study.

Limitations

There are several limitations of this study. With a single-centre study design, potential selection bias could not be controlled for. In addition, the heterogeneity of the study population can be seen as a limitation. Still, the nature of an all-comer study might be the strength of our design as it accurately reflects the patient population ICU physicians are confronted with on a daily basis. Due to the nature of the study with few exclusion criteria, a 24 h time window for the sample collection was established, since collection and analyses were not possible during the night-time. Although most samples were collected in a rather timely manner, a potential bias created by this 24 h window cannot be ruled out. Initial sample size calculation was done for the total cohort, leaving analyses in sub-groups potentially underpowered. However, the fact that non-survivors in the surgery/interventional groups displayed markedly elevated sNEP levels (median 660.2 pg/mL vs. 6532.6 pg/mL) may suggest biologic plausibility of the observed differences. Furthermore, due to the nature of an observational clinical study we cannot conclude any functional insights to NEP activity. Further studies are required to illuminate potential pathophysiological mechanisms involving NEP.

Conclusions

In summary, the present study comprises three major findings. First, within the total population, sNEP levels at the time of admission had no prognostic value regarding 30 day mortality. Second, patients undergoing cardiac surgery or catheter-based valve intervention showed significantly increased circulating levels of sNEP in 30 day non-survivors. Third, patients suffering from sepsis or septic shock as primary cause of admission displayed significantly increased levels of circulating NEP. These findings are in line with literature and could be explained through shedding by neutrophils. Our study might help in risk prediction for critically ill surgical/interventional patients and to shed some light on the involvement of sNEP in sepsis and septic shock.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Moss S, Subramanian V, Acharya KR. Crystal structure of peptide-bound neprilysin reveals key binding interactions. *FEBS Lett* 2020; **594**: 327–336.

2. Polna I, Aleksandrowicz J. Effect of adsorbents on IgM and IgG measles antibodies. *Acta Virol* 1975 Nov; **19**: 449–456.

3. Ronco P, Pollard H, Galceran M, Delauche M, Schwartz JC, Verroust P. Distribution of enkephalinase (membrane metalloendopeptidase, E.C.

3.4.24.11) in rat organs. Detection using a monoclonal antibody. *Lab Invest* 1988; **58**: 210–217.

4. Spillantini MG, Sicuteri F, Salmon S, Malfroy B. Characterization of endopeptidase 3.4.24.11 ("enkephalinase") activity in human plasma and cerebrospinal fluid. *Biochem Pharmacol* 1990; **39**: 1353–1356.

5. Erdös EG, Skidgel RA. Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. *FASEB J* 1989; **3**: 145–151.

6. Bayes-Genis A, Prickett TC, Richards AM, Barallat J, Lupón J. Soluble neprilysin retains catalytic activity in heart failure. *J Heart Lung Transplant* 2016; **35**: 684–685.

7. Pavo N, Prausmüller S, Bartko PE, Goliasch G, Hülsmann M. Neprilysin as a biomarker: challenges and opportunities. *Card Fail Rev* 2020; **6**: e23.

8. Pavo IJ, Pavo N, Kastner N, Traxler D, Lukovic D, Zlabinger K, Spannbauer A, Riesenhuber M, Lorant D, Bartko PE, Goliasch G, Hülsmann M, Winkler J, Gyöngyösi M. Heart failure with reduced ejection fraction is characterized by systemic NEP downregulation. JACC: basic to translational. *Science* 2020; **5**: 715–726.

9. Bayés-Genís A, Barallat J, Galán A, de Antonio M, Domingo M, Zamora E, Urrutia A, Lupón J. Soluble neprilysin is predictive of cardiovascular death and heart failure hospitalization in heart failure patients. *J Am Coll Cardiol* 2015; **65**: 657–665.

10. Bayes-Genis A, Barallat J, Galán A, de Antonio M, Domingo M, Zamora E, Gastelurrutia P, Vila J, Peñafiel J, Gálvez-Montón C, Lupón J. Multimarker strategy for heart failure prognostication. Value of neurohormonal biomarkers: neprilysin vs NT-proBNP. *Rev Esp Cardiol (Engl Ed)* 2015; **68**: 1075–1084.

11. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA* 1993; **270**: 2957–2963.

12. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; **13**: 818–829.

13. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996; **22**: 707–710.

14. Nashef SA, Roques F, Sharples LD, Nilsson J, Smith C, Goldstone AR, Lockowandt U. EuroSCORE II. *Eur J Cardiothorac Surg* 2012; **41**: 734–744; discussion 744-5 Epub 2012 Feb 29. PMID: 22378855.

15. Shahian DM, Jacobs JP, Badhwar V, Kurlansky PA, Furnary AP, Cleveland JC Jr, Lobdell KW, Vassileva C, Wyler von Ballmoos MC, Thourani VH, Rankin JS, Edgerton JR, D'Agostino RS, Desai ND, Feng L, He X, O'Brien SM. The Society of Thoracic Surgeons 2018 adult cardiac surgery risk models: Part 1-background, design considerations, and model development. *Ann Thorac Surg* 2018; **105**: 1411–1418 Epub 2018 Mar 22. PMID: 29577925.

16. Dixon J, Philips B. The interpretation of brain natriuretic peptide in critical care patients; will it ever be useful? *Crit Care* 2010; **14**: 184.

17. Lipinska-Gediga M, Mierzchala M, Durek G. Pro-atrial natriuretic peptide (pro-ANP) level in patients with severe sepsis and septic shock: prognostic and diagnostic significance. *Infection* 2012 Jun; **40**: 303–309.

18. Koch A, Voigt S, Sanson E, Dückers H, Horn A, Zimmermann HW, Trautwein C, Tacke F. Prognostic value of circulating amino-terminal pro-C-type natriuretic peptide in critically ill patients. *Crit Care* 2011; **15**: R45.

19. Potter LR. Natriuretic peptide metabolism, clearance and degradation. *FEBS J* 2011 Jun; **278**: 1808–1817.

20. Arrigo M, Vodovar N, Nougué H, Sadoune M, Pemberton CJ, Ballan P, Ludes PO, Gendron N, Carpentier A, Cholley B, Bizouarn P, Cohen-Solal A, Singh JP, Szymonifka J, Latremouille C, Samuel JL, Launay JM, Pottecher J, Richards AM, Truong QA, Smadja DM, Mebazaa A. The heart regulates the endocrine response to heart failure: cardiac contribution to circulating neprilysin. *Eur Heart J* 2018; **39**: 1794–1798.

21. Fielitz J, Dendorfer A, Pregla R, Ehler E, Zurbrügg HR, Bartunek J, Hetzer R, Regitz-Zagrosek V. Neutral endopeptidase is activated in cardiomyocytes in human aortic valve stenosis and heart failure. *Circulation* 2002; **105**: 286–289.

22. Fox AA, Marcantonio ER, Collard CD, Thoma M, Perry TE, Shernan SK, Muehlschlegel JD, Body SC. Increased peak postoperative B-type natriuretic peptide predicts decreased longer-term physical function after primary coronary artery bypass graft surgery. *Anesthesiology* 2011; **114**: 807–816.

23. Vodovar N, Séronde M-F, Laribi S, Gayat E, Lassus J, Januzzi JL, Boukef R, Nouira S, Manivet P, Samuel JL, Logeart D, Cohen-Solal A, Richards AM, Launay JM, Mebazaa A, GREAT Network. Elevated plasma B-type natriuretic peptide concentrations directly inhibit circulating Neprilysin activity in heart failure. *JACC Heart Fail* 2015; **3**: 629–636.

24. Morisaki T, Goya T, Ishimitsu T, Torisu M. The increase of low density subpopulations and CD10 (CALLA) negative neutrophils in severely infected patients. *Surg Today* 1992; **22**: 322–327.

25. Martens A, Eppink GJ, Woittiez AJ, Eidhof H, de Leij LF. Neutrophil function capacity to express CD10 is decreased in patients with septic shock. *Crit Care Med* 1999; **27**: 549–553.

26. Mebazaa A, Geven C, Hollinger A, Wittebole X, Chousterman BG, Blet A, Gayat E, Hartmann O, Scigalla P, Struck J, Bergmann A, Antonelli M, Beishuizen A, Constantin JM, Damoisel C, Deye N, Di Somma S, Dugernier T, François B, Gaudry S, Huberlant V, Lascarrou JB, Marx G, Mercier E, Oueslati H, Pickkers P, Sonneville R, Legrand M, Laterre PF, AdrenOSS-1 study investigators. Circulating adrenomedullin estimates survival and reversibility of organ failure in sepsis: the prospective observational multinational Adrenomedullin and Outcome in Sepsis and Septic Shock-1 (AdrenOSS-1) study. *Crit Care* 2018; **22**: 354.

27. Arfsten H, Goliasch G, Bartko PE, Prausmüller S, Spinka G, Cho A, Novak J, Haslacher H, Strunk G, Struck J, Hülsmann M, Pavo N. Increased concentrations of bioactive adrenomedullin subsequently to angiotensin-receptor/neprilysin-inhibitor treatment in chronic systolic heart failure. *Br J Clin Pharmacol* 2020; **87**: 916–924.