Rev Esp Cardiol. 2015;68(12):1053–1055

Editorial

Plasma Neprilysin Concentrations: A New Prognostic Marker in Heart Failure?

Concentraciones plasmáticas de neprilisina: ¿un nuevo marcador pronóstico en la insuficiencia cardiaca?

A. Mark Richards*

Christchurch Heart Institute, University of Otago, Christchurch, New Zealand, and Cardiovascular Research Institute, National University of Singapore, Singapore

Article history:
Available online 5 November 2015

Neprilysin, also known as neutral endopeptidase (NEP), endopeptidase 24.11, CD10, enkephalinase, and common acute lymphoblastic leukemia antigen (CALLA), is a membrane-bound metalloendopeptidase (EC 3.4.24.11) that has recently sprung to renewed prominence as a target in cardiovascular therapeutics. Numbered among the many substrates to NEP are multiple vasoactive peptides, including the cardiac natriuretic peptides, with important roles in regulating pressure and volume status in health and disease.[1,2] In the 1990s, NEP inhibitors were combined with angiotensin converting enzyme inhibitor (ACEI) therapy with the hope of adding the expected multifaceted benefits of enhanced plasma and tissue natriuretic peptide levels to the proven benefits of ACEI-related suppression of adverse renin-angiotensin-aldosterone system activation. After initial promise from trials in hypertension and heart failure, drug development was halted due to an unacceptable incidence of angioneurotic edema.[3,4] The current renewed interest in NEP reflects the recent success of a new combination, that of inhibition of NEP plus angiotensin II type 1 receptor blockade. The PARADIGM trial tested the NEP inhibitor sacubitril combined with the angiotensin receptor blocker valsartan (LCZ696) in chronic heart failure with reduced ejection fraction. The new treatment was significantly beneficial with respect to all important clinical endpoints and was associated with no excess of important adverse effects and in fact caused less renal failure and hyperkalemia than established evidence-based treatment with the ACEI enalapril.[5] The results point to this strategy being the greatest advance in the pharmacotherapy of chronic heart failure in the last 20 years.

In addition to its widespread tissue-based, membrane-bound form, NEP exists in a circulating nonbound form that retains catalytic activity.[6] In the article published in *Revista Española de Cardiología*, Bayes-Genis et al[7,8] build on previous work to confirm their original finding that plasma NEP concentrations have biomarker value in offering independent prognostic information in a cohort of patients with chronic heart failure. The presence of NEP activity in plasma has been recognized for more than 20 years and preliminary reports indicated that NEP activity may be altered in cardiovascular disease.[6] However, the relationship of plasma NEP concentrations to outcomes in heart failure was only recently reported by Bayes-Genis et al[7] in an ambulatory cohort of over 1000 attendees (recruited over several years) at a multidisciplinary heart failure clinic. Neutral endopeptidase concentrations independently predicted the composite endpoint of cardiovascular death or heart failure hospitalization in a comprehensive multivariate model adjusted for age, sex, ischemic pathogenesis of heart failure, left ventricular ejection fraction, New York Heart Association class, diabetes, hemoglobin, serum sodium, estimated glomerular filtration rate, and treatment (ACEI, angiotensin receptor blockers, and beta blockade) and including the benchmark biomarker N-terminal pro-B-type natriuretic peptide (NT-proBNP).

In the current report, analyses have been conducted in a subgroup of 797 of the original cohort with the focus on the biomarker performance of plasma NEP compared with NT-proBNP. With a sample of nearly 800 patients and with 300 primary endpoints occurring over several years of follow up the data set is well-powered to explore univariate and multivariate relationships between markers and outcomes. Both NEP and NT-proBNP were univariately related to age and ST2 with NEP exhibiting the stronger association with age and NT-proBNP more closely correlated to ST2. However, in sharp contrast to NT-proBNP, plasma NEP was not correlated with estimated glomerular filtration rate, blood urea, body mass index, left ventricular ejection fraction or high-sensitivity cardiac troponin T. Neutral endopeptidase, but not NT-proBNP, remained independently predictive of both the composite endpoint and cardiovascular death in a model incorporating the same variables as listed from the earlier report with the addition of heart rate, systolic blood pressure, and 2 further markers, ST2 and cardiac troponin T, measured by a high sensitivity assay. Receiver operator curve analyses show little effect of serial addition of NT-proBNP and then NEP to the base clinical predictive model for the composite endpoint or cardiovascular death. The authors comment that both NEP and NT-proBNP "showed good calibration and similar

SEE RELATED ARTICLE:
http://dx.doi.org/10.1016/j.rec.2015.07.001, Rev Esp Cardiol. 2015;68:1075–84.

* Cardiac Department, Cardiovascular Research Institute, National University of Singapore, 1E Kent Ridge Road, NUHS Tower Block, Level 9, NUHCS, Singapore 119228, Singapore.
Department of Medicine, University of Otago, Christchurch, PO Box 4345, Christchurch Hospital, Riccarton Avenue, Christchurch 8014, New Zealand.
E-mail addresses: mark.richards@cdhb.govt.nz, mdcarthu@nus.edu.sg

http://dx.doi.org/10.1016/j.rec.2015.09.004

discrimination and reclassification for both neurohormonal biomarkers, but only soluble neprilysin improved overall goodness-of-fit."[8]

How are we to interpret these results and what do they portend for the use of NT-proBNP and NEP as markers in the future? These findings are of great interest but raise many questions and require extended corroboration. They are so far confined to observations in a single cohort of heart failure patients with the current report derived from a subgroup of those included in the previously published paper.[7,8] First, is there a systematic change in plasma NEP concentrations in heart failure compared with health? The data from the current report and the earlier report from Bayes-Genis et al[7,8] do not address this matter and separation of marker levels in health from those in disease is a fundamental expectation of a biomarker.[7,8] Recently Vodovar et al[9] reported plasma NEP concentrations in patients presenting with breathlessness. The highest plasma concentrations of NEP occurred in chronic heart failure, the lowest in patients with noncardiac dyspnea, while levels in acute decompensated heart failure fell between. This differs from B–type natriuretic peptide/NT-proBNP, which exhibit their most extreme elevation in acute decompensated heart failure. Furthermore, although differing significantly, the groups exhibited a far smaller spread of NEP values between groups than that seen for NT-proBNP in analogous comparisons. Vodovar et al[9] also showed no relationship between NEP concentration and NEP activity. Instead, they reported a striking inverse relationship between NEP activity and B–type natriuretic peptide levels and provided supplementary in vitro evidence that B–type natriuretic peptide exceeding approximately 1000 pg/mL actively inhibits NEP activity. Hence, we have a major knowledge gap and uncertainty with respect a: *a)* how NEP concentrations alter between health and disease states and *b)* the relative importance of NEP concentration vs activity.

Notably, NEP and NT-proBNP did not correlate at all across the sample ($r = 0.01$; $P = .68$). This disjunction is puzzling given the incontrovertible prognostic power of NT-proBNP now supported over decades of reports from multiple cohorts in varied clinical settings.[10] If both markers are clearly prognostic, it is hard to understand why at least some weak correlation is not observed. This may be partly explained by the lack of reliable readings by the NEP assay below 250 pg/mL meaning that over 12% appear in a flat or artefactually "squashed" distribution at the lower range of samples. This may limit the visible spectrum of NEP values, preventing observation of a correlation between NT-proBNP and NEP. Resolution of this issue awaits development of more sensitive NEP assays with a greater range of reliable measurement.

The authors suggest that, due to its central biological position in so many relevant pathways, NEP may represent the best indicator of global neurohormonal activation. However, lack of correlations with NT-proBNP, ST2, and high-sensitivity cardiac troponin T mitigate against this concept. It has been long known that, in decompensated heart failure, NT-proBNP and other natriuretic peptides rise in parallel with concurrent activation of sympathetic and renin-angiotensin-aldosterone systems, as indicated by plasma catecholamines, renin, angiotensin 2, and aldosterone levels. Furthermore, endothelin 1 and angiotensin 2 cross talk with natriuretic peptide expression to elevate plasma natriuretic peptide levels for any given level of intracardiac pressures.[11] Hence, elevation of NT-proBNP is strongly suggestive of generalized neurohormonal activation in heart failure. We have no such data for plasma NEP concentrations at this stage.

How robust are the comparisons between NEP and NT-proBNP offered in this article? Interestingly, in the original article the tabled result for multivariable cox regression for risk of the primary composite endpoint and for cardiovascular death includes both NEP and NT-proBNP alongside age, sex, ischemic etiology of heart failure, left ventricular ejection fraction, New York Heart Association class, estimated glomerular filtration rate, diabetes, ACEI or ARB therapy, beta-blocker therapy, sodium, and hemoglobin. Both NEP and NT-proBNP were robustly and independently predictive of both endpoints. However, hazard ratios were larger (and associated P values were more significant) per standard deviation shift in NT-proBNP (hazard ratio for cardiovascular death and heart failure readmission, 1.32; $P < .001$; for cardiovascular death 1.43; $P < .001$) than for NEP (hazard ratio for cardiovascular death and heart failure readmission, 1.18; $P = .001$; for cardiovascular death, 1.18; $P = .006$). In contrast, in the current report, after ST2 and high-sensitivity cardiac troponin T are added to the same multivariate model (along with heart rate and systolic blood pressure) it is reported that NEP remains significant while NT-proBNP does not. However, the tabled models (see Tables 2 and 3 in Bayes-Genis et al[8]) do not include NEP and NT-proBNP simultaneously and the "significant" P values attributed to NEP are weak for both endpoints ($P = .03$ and .04 for the primary composite endpoint and cardiovascular death, respectively). Are the data presented like this because both markers "fall out" of the model when both are included? Notably, many model components (left ventricular ejection fraction, estimated glomerular filtration rate, body mass index, high-sensitivity cardiac troponin T) correlate directly or inversely with NT-proBNP to some degree but not NEP. When a model is heavily populated with correlated variables the vagaries of chance may readily reduce one or other variable from statistical significance without necessarily being a reliable (ie, consistent) finding or reflecting biological relevance. The findings need corroboration in further independent data sets.

In summary, the reported association of plasma NEP concentrations with outcome in a cohort with chronic heart failure is interesting. The current findings require robust corroboration. It is necessary to establish the dynamic response of plasma NEP to physiological and pathophysiological stimuli in health and disease and to explore in greater depth the relationship of plasma NEP to concurrent proven neurohormonal indicators. The relationship between NEP concentrations and NEP activity and its potential perturbation by disease states and drug treatment require elucidation. The relevance or not of baseline NEP concentrations or NEP activity to clinical response to NEP inhibiting drugs also remains unknown. With the arrival of a new therapeutic option in heart failure that incorporates inhibition of this pivotal catalyst we can be sure there will be a wealth of informative original research emerging on NEP in coming years.

CONFLICTS OF INTEREST

None declared.

REFERENCES

1. Skidgel RA, Erdos EG. Angiotesnin converting enzyme (ACE) and neprilysin hydrolyse neuropeptides: a brief history, the beginning and follow-ups to early studies. Peptides. 2004;25:521–5.
2. Turner AJ, Isaac RE, Coates D. The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. Bio Essays. 2001;23:261–9.
3. McClean DR, Ikram H, Garlick AH, Richards AM, Nicholls MG, Crozier IG. The clinical, cardiac, renal, arterial and neurohormonal effects of Omapatrilat, a vasopeptidase inhibitor, in patients with chronic heart failure. J Am Coll Cardiol. 2000;36:479–86.
4. Packer M, Califf RM, Konstam MA, Krum H, McMurray JJ, Rouleau JL, et al.; for the OVERTURE Study group. Comparison of omapatrilat and enalapril in patients with chronic heart failure. Circulation. 2002;106:920.
5. McMurray JJ, Packer M, Ashkay SD, Gong J, Lefkowitz MP, Rizkala AR, et al.; for the PARADIGM-HF Investigators and Committees. Angiotensin-neprilysin inhibition versus enalapril in heart failure. N Engl J Med. 2014;371:993–1004.

6. Yandle TG, Richards AM, Smith MW, Charles C, Livesey JH, Espiner EA. Assay of endopeptidase-24.11 (EC3.4.24.11) activity in human plasma. Application to in vivo studies of endopeptidase inhibitors. Clin Chem. 1992; 38:1785–91.
7. Bayes-Genis A, Barallat J, Galan A, de Antonio M, Domingo M, Zamora E, et al. Souble neprilysin is predictive of cardiovascular death and heart failure hospitalization in heart failure patients. J Am Coll Cardiol. 2015;65: 657–65.
8. Bayes-Genis A, Barallat J, Galán A, de Antonio M, Domingo Mar, Zamora E, et al. Estrategia multimarcador para estratificar el pronóstico en insuficiencia cardiaca. Valor de los marcadores neurohumorales: neprilisina frente a NT-proBNP. Rev Esp Cardiol. 2015;68:1075–84.
9. Vodovar N, Seronde MF, Laribi S, Gayat E, Lassus J, Jnauzzi JL, et al.; GREAT network. Elevated plasma B-type natriuretic peptide concentrations directly inhibit circulating neprilysin activity in heart failure. JACC Heart Fail. 2015;3:629–36.
10. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey Jr DE, Drazner MH, et al. American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2013;62:147–239.
11. Rademaker MT, Charles CJ, Espiner EA, Frampton CM, Nicholls MG, Richards AM. Combined inhibition of angiotensin II and endothelin suppresses the brain natriuretic peptide response to developing heart failure. Clin Sci. 2004;106:569–76.