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Review Article

Soluble neprilysin: A versatile biomarker for heart failure, cardiovascular diseases and diabetic complications—A systematic review

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ABSTRACT

The potential role of soluble neprilysin (sNEP) as a biomarker has been poorly documented. Hence, the present systematic review emphasizes to explore sNEP as an emerging biomarker for heart failure (HF), cardiovascular diseases, diabetic kidney diseases, and so on. A systematic review was performed using an online database search in PubMed, Science Direct, Scopus, and Cochrane Library. Articles reporting biomarker's performance to diagnose various diseases in human participants were included. The results of the search outcome were 4723 articles. Based on the inclusion criteria of the systematic review, finally, 12 articles fulfilled the selection criteria. In these studies, 8 cohort study, 2 cross-sectional study, 1 case–control, and 1 prospective cohort study were identified. All these studies clearly suggested sNEP as a potential biomarker for diagnosis of various diseases (HF, cardiovascular diseases, diabetic kidney diseases, metabolic syndrome). sNEP may be a potential biomarker for HF, cardiovascular diseases, diabetic kidney disease, and so on.

1. Introduction

Neprilysin (NEP) is a zinc-dependent membrane metallopeptidase with a molecular weight of 90 kDa and contains glycosylation sites.[1] NEP is highly conserved in mammals, and there is only a 6-amino-acid variance in sequences between humans and rats.[2] It is recognized by several names as enkephalinase, neutral endopeptidase, common acute lymphoblastic leukemia antigen, endopeptidase 24.11, and CD10.[3,4] NEP is a familiar enzyme that was defined and fully characterized several decades ago. It is abundant and expressed by the highest concentrations in the proximal tubules and also expressed in the kidneys, lungs, endothelial cells, vascular smooth muscle cells, cardiac cells, fibroblasts, neutrophils, adipocytes, testes, and brain. NEP is also relatively indiscriminate in the cardiovascular system, and NEP cleaves numerous vasoactive peptides and others. Studies have documented an alternative processing form of NEP, soluble NEP (sNEP), which exists in the plasma and urine.[5,6] sNEP has an enzymatic activity to degrade peptides equally to membrane-bound NEP.

The brush border of proximal tubular cell expression of NEP, the most susceptible elasticity of epithelial cells, was one of the main reasons to start testing its existence in urine.[7] In addition to this protein, many other proteins were also in urinary exosomes.[8] Furthermore, its contribution to blood pressure regulation and the development of inhibitors has fetched this protein to the prime of medical interest.[9,10] Then, findings of the wide distribution of NEP and its unexpectedly extensive potentially important roles for the endopeptidase in cardiovascular, renal, pulmonary, gastrointestinal, and neurological functions.

Furthermore, an insufficient attempt was made to evaluate the sNEP as a biomarker for heart failure (HF), cardiovascular disease, kidney disease, and so on. The purpose of the present systematic review was to provide the cumulative research output about whether sNEP really plays a key role as a versatile biomarker for diagnosing various diseases.

2. Materials and methods

2.1. Literature search strategies for identification of relevant studies

The literature search was conducted in PubMed, Scopus, Science Direct, Google Scholar, and Cochrane Library using the following

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keywords: 'Neprilysin' plus 'biomarker' without language restriction. The searches were performed independently by 2 investigators. We have to screen all the abstracts available in English. Abstracts are manually screened based on the eligibility criteria and exclusions cross-checked by a member of the team. Any studies that fulfill the inclusion criteria were then to be reviewed in full. Full-text articles will be screened independently by two investigators for eligibility. Disagreement about any article's eligibility will be resolved by consent. All suggested articles will be screened for eligibility by using the same criteria as for the original articles. If necessary, we will revise the literature search to find articles similar to those missed in the original search.

2.2. Study inclusion and exclusion criteria

The systematic review considered the following inclusion criteria: (1) full research articles in English, (2) articles published after 2009, (3) human participants in both gender with sample size more than 20, and (4) assessment of sNEP as a biomarker are considered. Similarly, the exclusion criteria included the following: (1) abstracts and conference papers, (2) articles in other than the English language, (3) other than human studies such as animal and computational studies, (4) tissue expression studies about NEP, (5) human participants with sample size less than 20, and (6) articles published before 2009.

2.3. Data abstraction and data management

Each study will be extracted by one skilled scientist. The extraction will be reviewed and confirmed by at least one other methodologist. Any disagreements will be resolved by discussion among the team. Data will be extracted into standard forms.

2.4. Data synthesis

All included studies will be summarized in tables that tabulate the study populations, study design, biomarkers evaluated, and outcomes of the studies.

3. Results and discussion

Two reviewers independently completed the selection and review of articles. Full-text articles found in the initial search were cross-checked with the reference. Studies that satisfied the full-text article selection criteria included the following: (1) primary research, (2) a sample size of $\geq$20 with any disease, (3) assessment of sNEP as a biomarker, (4) full-text article in English, (5) a general population (e.g., not a single gender), and (6) studies carried out after 2009. The systematic review search yielded about 4722 unique references from four databases (Fig. 1). All the articles based on inclusion criteria were reviewed and the duplication was removed. The studies published before the year 2009 were excluded from the review. After screening the abstracts, 12 articles met the selection criteria (Table 1).

3.1. Association among soluble neprilysin, HF, and cardiovascular disease

The circulating sNEPhas recently emerged as a potential biomarker for the prognosis of cardiovascular death and hospital admission for patients with acute and chronic HF.

The factors which determine the readmission in the hospital for acute HF are not well described. However, the risk of recurrent hospitalization is significantly increased in these patients. To address this problem, Núñez et al[11] conducted an experimental study to evaluate the association between sNEP and the risk of long-term repeated hospitalizations in a cohort of patients with AHF, because, NEP (NEP) is an enzyme with a pivotal role in the pathophysiology of HF. In their study, 210 patients with AHF were enrolled, and unplanned hospital admission was the primary end point. The decision on the clinical management of the primary end point was based on sNEP levels. Finally, sNEP was found to be significantly associated with the risk of any rehospitalization. Another interesting cohort study has emphasized the direct comparison of sNEP with existing markers for HF to establish the prognostic marker. Seven hundred ninety-seven consecutive ambulatory patients with HF participated and were followed up for 4.7 years. A primary end point has been cardiovascular death or HF hospitalization. A secondary end point explored cardiovascular death alone. sNEP remained independently associated with both the end points than other biomarkers and concluded that sNEP is a novel independent prognostic biomarker for HF.[12]

Elevated sNEP levels predicted as an increased risk of recurrent all-cause, cardiovascular, and AHF admissions in outpatients with HF have been revealed in the study of 1021 consecutive ambulatory patients with HF with a median follow-up period of 3.4 years. The conclusion points were the number of all-cause, cardiovascular, and AHF hospitalizations during follow-up.[13]

Arrigo et al[14] measured sNEP concentration at admission and before discharge in 50 patients admitted for acute dyspnea. They confirmed that increased sNEP has been observed in immediate improvement after acute dyspnea or significant improvement after total artificial heart transplantation. Another study also observed from 98 patients with HF sNEP as a potential biomarker and also as a biotarget in HF.[15]

3.2. Association of neprilysin with type 2 diabetes mellitus and kidney function

Chronic kidney disease (CKD), which is considered by an advanced deterioration in the glomerular filtration rate over more than 3 months, is a devastating disease and is frequently accompanied by albuminuria. The fundamental mechanism of the development of diabetic kidney disease to end-stage renal disease is not fairly understood. In routine clinical practice, kidney diseases do not have any symptoms and biomarkers at its early stages.[16] Hence, the evaluation of new biomarkers for the early detection and management of diabetic CKD is inevitable.

A cross-sectional study was performed on 60 patients with microalbuminuria and macroalbuminuria. Patients were categorized as nondiabetic (ND) and diabetic with normoalbuminuria, microalbuminuria, and macroalbuminuria. In their study, angiotensin 2 (ACE2) and NEP were evaluated in all the groups and concluded that ACE2 and NEP as noninvasive biomarkers to evaluate diabetic kidney disease progression at an early stage.[17] Guillén-Gómez et al[18] conducted a similar kind of study in 21 patients with diabetic nephropathy. However, they adopted the urinary proteome analysis to identify biomarkers of a clinical outcome, and tandem mass tag has been used for quantification of biomarkers. Finally, the observation revealed that NEP and VCAM-1 are promising biomarkers in the diagnosis and therapeutic management of DN.

Acute kidney injury (AKI) is one of the complications observed in critically ill patients who are in the intensive care units which causes predominantly sepsis. The new potential biomarker screening is extremely important to avoid complications due to AKI. Pajenda et al[19] carried out a research study to address the aforementioned problem which included 90 patients who are admitted to the intensive care units. The urinary NEP has been estimated in all the patients and compared them with 55 healthy

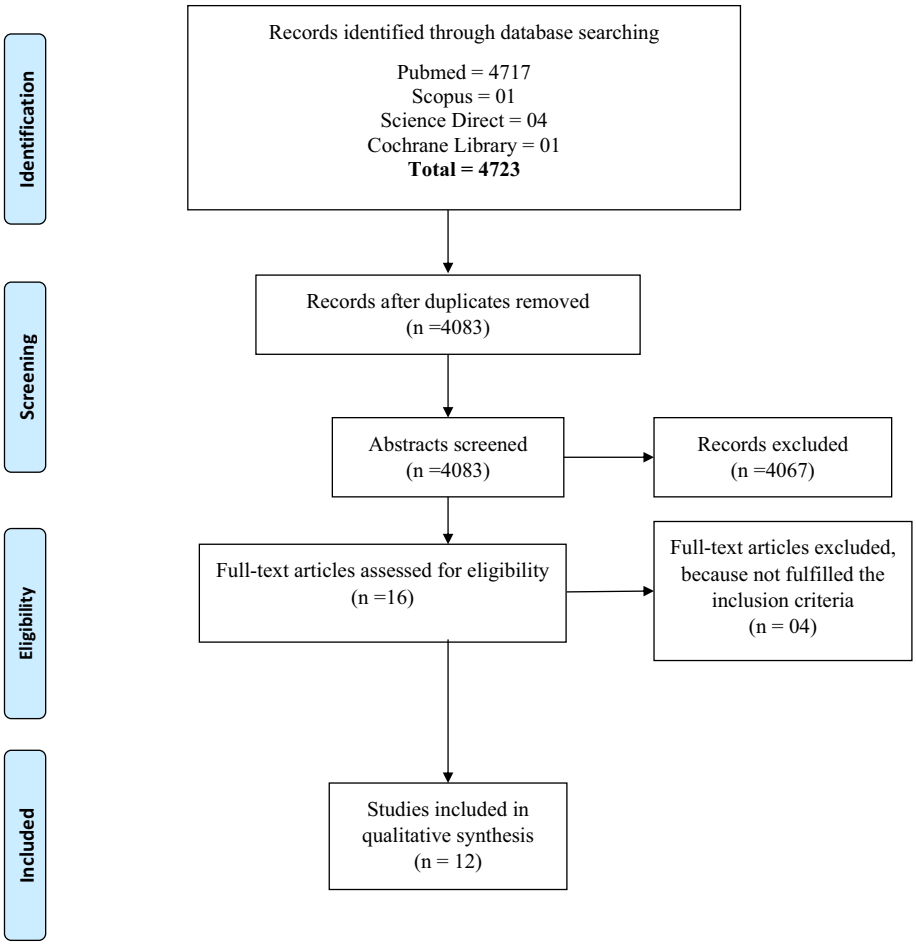

Fig. 1. PRIMA 2009 flow diagram.

controls. They confirmed that raised urinary NEP is suggestive of proximal tubular cell stress or injury.

3.3. Miscellaneous

3.3.1. Cardiac arrest

Medical management of postcardiac arrest may be challenging; its mortality rate remains high[20] and is characterized by myocardial dysfunction.[21] Besides, early risk identification is an urgent need for patients with out-of-hospital cardiac arrest (OHCA). In this setting, biomarkers are the prognostic tool that assists to understand pathophysiological processes and support in decision-making.[22] Zelniker et al[23] sought to study the potential prognostic role of sNEP to understand the pathophysiology in patients with OHCA. One hundred forty-four patients with the successful return of natural circulation after OHCA of nontraumatic origin were enrolled, and sNEP was measured. They were followed up at least 30 days, and the primary end point was time to all-cause mortality. This study confirmed that sNEP was independently associated with all-cause mortality in patients with OHCA of nontraumatic origin and useful in the stratification of risk in these patients.

3.3.2. Obesity

NEP is produced by adipocytes,[24] predicted that it might have a key role as an adipokine regulating characteristics of adipocyte function. Inhibition of NEP in obese insulin-resistant Zucker rats improved whole-body insulin-mediated glucose disposal and dual Angiotensin-converting enzyme (ACE)/NEP inhibition induced deep insulin sensitization and improved myocardial glucose uptake.[25–27] It proved that NEP directly involved in the development of insulin resistance. Standeven et al[28] conducted an interesting study to investigate the association of insulin resistance between NEP activity, obesity and components of the metabolic syndrome in 318 healthy individuals characterized for insulin resistance using the homeostasis model assessment and the presence of the metabolic syndrome (MetS) according to the International Diabetes Federation (IDF) definition. They concluded that increased plasma NEP activity associated significantly with obesity and the development of insulin resistance.

3.3.3. General population

Reddy et al[29] have conducted a large community-based cohort study in the general population with 1536 participants. This study is unique because there are no such studies conducted previously. They measured plasma sNEPalong with natriuretic peptide and assessed the ventricular structure. The conclusion from this study is that there was no correction between NEP and natriuretic peptides. This result was contradicted in previous studies. However, this study has been conducted on the general population which should also be considered.

3.3.4. Pathophysiology of NEP

NEP is extensively scattered in several tissues, which include the kidney, lung, brain, heart, and vasculatures. Notably, the kidney is

Table 1
Summarized studies included in the review.

Reference	Study population	Biomarkers analyzed	Analytical method used for neprilysin estimation	Brief conclusion of the study	Type of study
A. Bayes-Genis et al, 2015	797 patients with HF	Plasma sNEP, NT-pro BNP, hscTnT, ST2	Modified sandwich immunoassay	sNEP independently associated with HF-related death than NT-pro BNP	Cohort
Thomas A Zelniker et al, 2018	144 patients of cardiac arrest of nontraumatic origin out-of-hospital	Plasma sNEP, hsTnT, hsCRP, NT-pro BNP	Human neprilysin DuoSet ELISA research kit	sNEP independently associated with all-cause mortality in patients with out-of-hospital cardiac arrest of nontraumatic origin	Cohort
Pajenda et al, 2017	90 critically ill patients	Urine NEP	Human NEP ELISA	Elevated urinary NEP is indicative of proximal tubular cell stress or injury.	Cohort
Sridevi Gutta et al, 2018	60 patients with type 2 DM with a history of microalbuminuria and macroalbuminuria	ACE2 and NEP	Human ELISA kit	ACE2 and NEP as noninvasive biomarkers to assess kidney damage in patients with diabetes at an early stage.	Cross-sectional
Mattia Arrigo et al, 2018	50 patients (28 patients with acute HF and 22 acute noncardiac dyspnea)	sNEP	Modified sandwich immunoassay	Short-term clinical improvement after acute dyspnea or mid-term improvement after total artificial heart transplantation are associated with an increase rather than a decrease in sNEP	Cohort
Antoni Bayes-Genis et al, 2016	98 patients with chronic HF	sNEP	Fluorometric assay	Circulating sNEP as a biotarget in heart failure	Cohort
Antoni Bayés-Genís et al, 2015	1069 patients with HF	sNEP	Modified sandwich immunoassay	High levels of neprilysin are found in the circulation of patients with HF and that neprilysin concentrations are indicators of adverse outcomes for both cardiovascular mortality and morbidity.	Cohort
Julio Nu ~ nez et al, J 2017	1021 consecutive ambulatory patients with heart failure	sNEP, NT-pro BNP	Modified sandwich immunoassay	Elevated sNEP levels predicted an increased risk of recurrent all-cause, cardiovascular, and AHF admissions in ambulatory patients with heart failure.	Cohort
Elena Guillén-Gómez et al, 2018	21 patients with type 2 diabetic [12 without DN (control patients) and 9 with incipient DN (DN basal)]	Urine NEP, VCAM-1	Specific enzyme-linked immunosorbent assay	Neprilysin and VCAM-1 as potential new tools as DN progression biomarkers	Case control
Kristina F. Standeven et al, 2011	318 clinically healthy white men of European origin, characterized for the presence of the MetS	sNEP, tissue plasminogen activator, insulin, plasminogen activator inhibitor-1	Modified fluorescence	Obesity and the development of insulin resistance is associated with increased plasma NEP levels	Cross-sectional
Julio Núñez et al, 2016	210 patients consecutively admitted for AHF	NT-pro BNP, hscTnT, sNEP	Modified sandwich immunoassay	sNEP was associated with the risk of long-term recurrent all-cause and AHF rehospitalizations	Prospective cohort
Reddy et al, 2019	1536 patients in the general population	sNEP, ANP, BNP, NT-pro ANP, NT-pro BNP	Sandwich ELISA	sNEP and natriuretic peptide did not correlate.	Large community-based cohort study

HF = heart failure; sNEP = soluble neprilysin; NT-pro BNP = N-terminal brain natriuretic peptide; hscTnT = high sensitive cardiac troponin T; ST2 = suppression of tumorigenicity 2; NEP = neprilysin; ACE2 = angiotensin-converting enzyme 2; IL6 = interleukin; VCAM-1 = human vascular cell adhesion molecule-1; STEMI = ST-segment elevation myocardial infarction; DM = diabetes mellitus; DN = diabetic nephropathy; MetS = metabolic syndrome; AHF = acute heart failure; ANP = A-type natriuretic peptide; BNP = brain natriuretic peptide; NT-pro ANP = N-terminal pro-ANP; NT-pro BNP = N-terminal pro-BNP.

the richest source, which was identified with the use of a NEP monoclonal antibody in porcine renal tissues.[30] A critical characteristic of NEP is that it cleaves and degrades a range of bioactive peptides. From this perception, NEP has high significance to cardiovascular and renal regulation, and accepting the modulations of these substrates by NEP is critical for understanding therapeutic and diagnostic associations.

NEP is responsible for the degradation of endogenous natriuretic peptides that have important adaptive cardiovascular effects, including direct effects to inhibit the renal sodium reabsorption, suppress the secretion of aldosterone from the adrenal gland,[31–33] and inhibit cardiac inflammation and fibrosis.[34–36] The amount of NEP is raised in chronic HF, increased further during acute decompensation, and is connected with a contrary outcome in patients with a reduced ejection fraction.[6,13,37] NEP inhibition has positive effects on cardiac remodeling[35,38,39] and decreases the morbidity and mortality of patients with HF with decreased systolic function.[40]

NEP is expressed on the surface of mature adipocytes[24,41] which seemingly shed the enzyme into the plasma where its soluble form can be measured. Consumption of a high-fat diet raises circulating levels of NEP, and visceral fat contains high levels of the enzyme.[41–43] Moreover, the improved renal sympathetic nerve activity that describes patients with obesity leads to stimulation of NEP within the kidney.[34] People with obesity have increased levels of NEP in proportion to their body mass,[42,43] and circulating levels of sNEP are predominantly elevated in patients with obesity with HF with a preserved ejection fraction.[44]

4. Conclusion

In conclusion, the present systematic review validates that measurement of sNEP, a versatile biomarker, seems to comparatively provide good insight into the diagnosis of HF, cardiovascular diseases, diabetic kidney disease, AKI, cardiac arrest, and obesity. Large cohort studies are needed to confirm our validation.

Ethics approval and consent to participate

Not applicable. This study was a systematic review.

Consent for publication

Not applicable.

Data statement

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

K.R. and G.P. contributed to research idea and study design; data acquisition and data analysis; manuscript drafting. K.R. contributed in mentorship. All authors approve the final version of the manuscript.

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