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Soluble Neprilysin Is Predictive of Cardiovascular Death and Heart Failure Hospitalization in Heart Failure Patients

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ABSTRACT

BACKGROUND Neprilysin is a membrane-bound enzyme that breaks down natriuretic peptides. The PARADIGM-HF (Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure) trial showed that patients with heart failure (HF) treated with an angiotensin receptor neprilysin inhibitor lived longer without being hospitalized for HF than those receiving standard care with enalapril.

OBJECTIVES This study sought to assess the presence of circulating soluble neprilysin in a real-life cohort of HF patients and correlate neprilysin levels with outcomes.

METHODS Circulating soluble neprilysin was measured with a modified sandwich immunoassay in consecutive ambulatory patients with HF who were followed up for 4.1 years. Associations between neprilysin level and a composite endpoint that included cardiovascular death or HF hospitalization were explored.

RESULTS Median neprilysin concentration in 1,069 patients was 0.642 ng/ml (median quartile 1 to 3: 0.385 to 1.219). Neprilysin weakly but significantly correlated with age (rho = 0.16; $p < 0.001$). In age-adjusted Cox regression analyses, neprilysin concentrations were significantly associated with the composite endpoint (hazard ratio [HR]: 1.17; 95% confidence interval [CI]: 1.06 to 1.29; $p = 0.001$) and cardiovascular death (HR: 1.19; 95% CI: 1.06 to 1.32; $p = 0.002$). In comprehensive multivariable analyses, soluble neprilysin remained significantly associated with both the composite endpoint (HR: 1.18; 95% CI: 1.07 to 1.31; $p = 0.001$) and cardiovascular death (HR: 1.18; 95% CI: 1.05 to 1.32; $p = 0.006$).

CONCLUSIONS Identification of circulating neprilysin in HF patients and the positive association of neprilysin with cardiovascular mortality and morbidity further support the importance of NEP inhibition for augmenting natriuretic peptides as a therapeutic target. (J Am Coll Cardiol 2015;65:657–65) © 2015 by the American College of Cardiology Foundation.

Neprilysin is known by a variety of other names, including neutral endopeptidase, CD10, enkephalinase, common acute lymphoblastic leukemia antigen (CALLA), and endopeptidase-24.11. Neprilysin catalyzes the degradation of several vasodilator peptides, including natriuretic peptides, angiotensin II, bradykinin, substance P, adrenomedullin, and endothelin-1 (1–3). Therefore, inhibiting neprilysin will augment the naturally occurring natriuretic peptides, which promote natriuresis, induce vasodilation, and reduce cardiac hypertrophy and fibrosis. Because neprilysin participates in the breakdown of angiotensin II, neprilysin inhibitors might increase circulating angiotensin II levels. This provides a rationale for agents that block both neprilysin and angiotensin II.

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LCZ696, an angiotensin receptor neprilysin inhibitor (ARNI), contains the neprilysin inhibitor prodrug AHU377 and the angiotensin II receptor antagonist valsartan (4). It has been tested successfully

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ABBREVIATIONS AND ACRONYMS

ACEI = angiotensin-converting enzyme inhibitor

ARB = angiotensin II receptor blocker

ARNI = angiotensin receptor neprilysin inhibitor

BNP = B-type natriuretic peptide

CI = confidence interval

eGFR = estimated glomerular filtration rate

HR = hazard ratio

HF = heart failure

HFrEF = heart failure with reduced ejection fraction

hsTnT = high-sensitivity troponin T

LVEF = left ventricular ejection fraction

NT-proBNP = N-terminal pro-B-type natriuretic peptide

NYHA = New York Heart Association

in patients with hypertension (5) and with heart failure (HF) with preserved ejection fraction (6). Early closure of the PARADIGM-HF (Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure) trial in patients with HF with reduced ejection fraction (HFrEF) (7) was recently announced because patients who received LCZ696 lived longer without being hospitalized for HF than those who received standard care with the angiotensin-converting enzyme inhibitor (ACEI) enalapril.

Neprilysin is a membrane-bound enzyme with a large extracellular catalytic domain, a single transmembrane region, and a short (27 amino acids) cytoplasmic N-terminal domain (8,9). Previous studies showed that neprilysin, like many other membrane-bound metalloproteases, can be released from the cell surface, producing a non-membrane-associated form that retains catalytic activity (10,11). Previous reports have identified neprilysin in biological fluids (12,13), but no evidence exists of circulating neprilysin as a pathobiological surrogate in patients with HF. Accordingly, our aim was to assess for the first time the presence of circulating soluble neprilysin in a real-life cohort of HF patients and to correlate neprilysin levels with outcomes.

METHODS

STUDY POPULATION. Ambulatory patients treated at a multidisciplinary HF clinic between May 22, 2006 and May 22, 2013 were consecutively included in the study. Referral inclusion criteria and blood sample collection have been described elsewhere (14). In summary, patients were referred to the HF clinic by cardiology or internal medicine departments and, to a lesser extent, from the emergency or other hospital departments. The principal referral criterion was HF according to the European Society of Cardiology guidelines irrespective of pathogenesis, at least 1 hospitalization for HF, or a reduced left ventricular ejection fraction (LVEF). Neprilysin and N-terminal prohormone B-type natriuretic peptide (NT-proBNP) were analyzed from the same blood sample stored at −80°C, without previous freeze-thaw cycles. All samples were obtained between 9 AM and noon.

All participants provided written informed consent, and the local ethics committee approved the study. All study procedures were in accord with the ethical standards outlined in the Helsinki Declaration of 1975, as revised in 1983.

FOLLOW-UP AND OUTCOMES. All patients were followed up at regular pre-defined intervals, with additional visits as required in case of decompensation. The regular visitation schedule included a minimum of quarterly visits with nurses, biannual visits with physicians, and elective visits with geriatricians, psychiatrists, and rehabilitation physicians (14,15). Patients who did not attend the regular visits were contacted by telephone.

The primary outcome was the composite of cardiovascular death or HF hospitalization. Secondary outcomes included cardiovascular and all-cause death. A death was considered of cardiovascular origin if it was caused by HF (decompensated HF or treatment-resistant HF, in the absence of another cause); sudden death (unexpected death, witnessed or not, of a previously stable patient with no evidence of worsening HF or any other cause of death); acute myocardial infarction (directly related in time with acute myocardial infarction, whether attributable to mechanic, hemodynamic, or arrhythmic complications); stroke (associated with recent acute neurological deficit); procedural (post-diagnostic or post-therapeutic procedural death); or other cardiovascular causes (e.g., rupture of an aneurysm, peripheral ischemia, or aortic dissection). HF hospitalizations were identified from clinic records, hospital wards, or electronic Catalan history record. Fatal events were identified from the clinical records of patients with HF, hospital wards, the emergency department, or general practitioners, or by contacting the patient's relatives. Furthermore, data were verified from the databases of the Catalan and Spanish Health Systems. Adjudication of events was performed by 2 of the authors (M.D and J.L.).

ASSAYS. Human neprilysin was measured with a modified sandwich immunoassay (human neprilysin/CD10 ELISA kit, Aviscera Bioscience, Santa Clara, California, code No. SK00724-01, lot No. 20111893). To improve the analytic sensitivity of the method and to obtain a lower limit of sample quantification, several modifications were made: 1) serum aliquots were diluted one-quarter in dilution buffer provided by the manufacturer (DB09) before incubation; 2) the kit was transferred to an automated robotic platform (Basic Radim Immunoassay Operator 2 [BRIO 2], Radim SpA, Pomezia, Italy) that performed all incubations at a constant temperature of 30°C, with 1,000 revolutions/min mixing; and 3) initial sample incubation was extended to 150 min to achieve a higher slope in the calibration curve and better assay sensitivity