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Clinical Investigation

HE4 Serum Levels Are Associated with Heart Failure Severity in Patients With Chronic Heart Failure

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ABSTRACT

Background: The novel biomarker human epididymis protein 4 (HE4) shows prognostic value in acute heart failure (HF) patients. We measured HE4 levels in patients with chronic heart failure (CHF) and correlated them to HF severity, kidney function, and HF biomarkers, and determined its predictive value.
Methods: Serum HE4 levels in patients (n = 101) with stable CHF with reduced left ventricular ejection fraction (LVEF <45%) from the Vitamin D CHF (VitD-CHF) study (NCT01092130) were compared with those in age- and sex-matched healthy control subjects (n = 58) from the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study.
Results: HE4 levels were higher in CHF compared with control subjects (69.2 pmol/L [interquartile range 55.6-93.8] vs 56.1 pmol/L [46.6-69.0]; $P < .001$) and were higher with increasing New York Heart Association functional class. Levels were associated with HF risk factors, including age, gender, diabetes, smoking and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP). HE4 demonstrated strong associations with kidney function and HF fibrosis biomarkers. In a multivariable model, we identified creatinine, NT-proBNP, galectin-3, high-sensitive troponin T, and smoking as factors associated with HE4. Independently from these factors, HE4 levels predicted death and HF rehospitalization (5-year follow-up, hazard ratio 3.8; confidence interval 1.31–11.1; $P = .014$).
Conclusions: HE4 levels are increased in CHF, correlate with HF severity and kidney function, and predict HF outcome. (*J Cardiac Fail 2017;23:12–19*)
Key Words: HE4, WFDC2, chronic heart failure, fibrosis.

Heart failure (HF) is a major health problem in the Western world. With increasing prevalence, high morbidity, and 5- and 10-year mortality of 50% and 90%, respectively, HF has enormous social and economic consequences within our society.[1] HF is usually preceded by various cardiovascular diseases, including myocardial infarction, hypertension, atrial fibrillation, and valvular diseases, that provoke structural, morphologic, and functional changes of the heart, commonly referred to as cardiac remodeling.[2,3]

HF is a clinical syndrome, but besides signs and symptoms, circulating HF biomarkers can be helpful in diagnosis of HF and could improve risk stratification and guide treatment, as has been shown for N-terminal prohormone of B-type natriuretic peptide (NT-proBNP).[4–6] Until now, NT-proBNP is the HF biomarker that is used for diagnostic and prognostic purposes and has been included in the HF guidelines of the European Society of Cardiology (ESC) and the guidelines of the American Heart Association (AHA).[7,8] New biomarkers under investigation have been shown to reveal underlying processes, and may potentially help in patient-tailored therapy. Examples include fibroblast growth factor 23 (FGF-23),[9] inflammatory biomarkers such as C-reactive protein and

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interleukin-6,[6] and fibrotic biomarkers such as galectin-3 (Gal-3)[6,10] and soluble suppression of tumorigenicity 2 (sST2).[11]

Recently, we reported that circulating human epididymis protein 4 (HE4) levels in acute heart failure (AHF) patients are correlated with HF severity and kidney function and were strongly predictive of HF outcome.[12] HE4, also known as whey acidic protein four–disulfide core domain 2 (WFDC2), was originally identified as a secretory protein present in the human epididymis.[13–15] Later studies showed expression of HE4 in multiple tissues throughout the body, including kidney, respiratory tract, and other organs.[13–15] HE4 levels are also elevated in patients with ovarian cancer,[16] and the use of these levels has been approved for monitoring epithelial ovarian cancer. Although the exact function of HE4 is not yet known, HE4 sequence shows similarity to proteinase inhibitors, which suggests a functional role of HE4 in fibrosis formation.[13,17] LeBleu et al showed that fibroblast-derived HE4 is a mediator of kidney fibrosis.[18] Matrix proteinase activity was inhibited by HE4, and degradation of type 1 collagen by matrix metalloproteinases was reduced. Other WFDC proteins have been associated with fibrosis formation and inflammatory processes.[17] Because these processes also play a pivotal role in pathologic cardiac remodeling, we decided to further investigate the association between HE4 levels and HF.

Previously we identified HE4 as a biomarker elevated in patients with AHF. In these patients, HE4 levels correlated with HF severity and kidney function and were predictive of HF outcome.[12] No data exist about HE4 and chronic heart failure (CHF). Therefore, in the present study, the association of HE4 levels with HF severity and other HF biomarkers and kidney function was investigated in patients with CHF. In addition, its predictive value regarding HF outcome was analyzed.

Methods

Study Population

VitD-CHF Study. The Vitamin D Chronic Heart Failure (VitD-CHF) study was designed to study the effects of vitamin D (VitD) supplementation on plasma renin activity (PRA) in patients with CHF with reduced ejection fraction. The VitD-CHF study and its methods have been described in detail elsewhere.[19] In short, patients with CHF with left ventricular ejection fraction (LVEF) ≤45% receiving HF treatment according to the ESC guidelines were enrolled in this study. Optimal medical therapy was defined as being treated with a β-blocker (unless contraindicated), an angiotensin-converting enzyme (ACE) inhibitor or, in the case of ACE inhibitor intolerance, an angiotensin receptor blocker (ARB), each on a stable dose. Estimated glomerular filtration rate (eGFR) <60 mL min^{-1} 1.73 m^{-2} was an exclusion cirterion. In total, 101 patients were included in this trial. HE4 levels were determined in baseline serum samples collected before treatment with VitD started. Serum samples were stored at −80°C until analysis and were available for all 101 patients. Patients were followed for 5 years, and a composite end point of all-cause mortality and HF rehospitalization was analyzed.

PREVEND. To compare HE4 levels between patients with and without CHF, an age- and sex-matched control group of non-HF subjects was composed from the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study. The PREVEND study was designed to prospectively investigate the natural course of microalbuminuria and the relation of microalbuminuria with future renal and cardiovascular diseases, as described in detail elsewhere.[20] In short, 85,421 subjects from the general population aged 28–75 years were sent a questionnaire with questions regarding cardiovascular risk factors and morbidity and a vial to collect an early-morning urine sample. In total, 40,856 subjects responded. Subjects with diabetes mellitus and pregnant women were excluded. Subjects with a morning urinary albumin concentration (UAC) >10 mg/L and a selection of subjects with a UAC <10 mg/L were included, a total of 8592 subjects. Serum samples were stored at −80°C until analysis. For the present study, a control group of non-HF subjects was created as follows: subjects with chronic heart disease and/or recorded cardiac events, including history of myocardial infarction, angina pectoris, coronary artery bypass grafting, and/or percutaneous coronary intervention, were excluded. Also, subjects with renal disease requiring dialysis and/or a creatinine clearance <60 mL/min were excluded. After exclusion, out of 4268 suitable control subjects, 58 age- and sex-matched subjects were randomly selected to form a suitable control group for the VitD-CHF cohort.

Both the VitD-CHF study and the PREVEND study were approved by the Ethics Committee of the University Medical Center Groningen and performed in accordance with the declaration of Helsinki. Every participant provided written informed consent.

Measurement of HE4

Serum HE4 levels were measured with the use of an enzyme-linked immunosorbent assay (ELISA; Fujirebio Diagnostics, Malvern, Pennsylvania). The Food and Drug Administration approved the use of this ELISA to monitor patients with ovarian cancer. This ELISA allows quantitative measurements of HE4 levels in serum and has a detection limit of ≤25 pmol/L and a coefficient of variance (CV) ≤15%. Standard curves and control samples were included in duplicate on all plates. The lower control (expected value 38–70 pmol/L, average measured value 55.33 ± 1.29 pmol/L) had a CV of 2.3%. The upper control (expected value 304–455 pmol/L, average measured value 404.71 ± 21.65 pmol/L) had a CV of 5.3%. Interassay reproducibility was 97.7%.

Laboratory Measurements and Definitions

On the day of the hospital visit for the VitD-CHF study, routine laboratory measurements were performed, including glycated hemoglobin (HbA_{1c}), total cholesterol, low-density lipoprotein (LDL), and albumin. NT-proBNP, creatinine, and high-sensitivity troponin T (hsTnT) were measured with the use of the Roche Modular system (Roche,

Mannheim, Germany). Gal-3 was measured with the use of an ELISA (BG Medicine, Waltham, Massachusetts). Plasma aldosterone was measured with the use of a solid-phase I-125 radioimmunoassay (Siemens Diagnostics, The Netherlands). Serum collagen type 1 cross-linked telopeptide (ICTP) was measured with the use of a quantitative enzyme immunoassay (UniQ ICTP, Orion Diagnostica, Espoo, Finland). Serum protocollagen 3 N-terminal propeptide (PIIINP) was measured with the use of a quantitative radio immunoassay (UniQ PIIINP; Orion Diagnostica). PRA was measured with the use of an indirect radioimmunoassay kit for quantitative determination of angiotensin I (Cisbio International, Codolet, France). Plasma FGF-23 was measured with the use of an ELISA (Immutopics, San Clemente, California). Body mass index (BMI) was calculated by dividing weight by height squared. Stroke volume (SV) was calculated by subtracting left ventricular end-systolic volume (LVESV) from left ventricular end-diastolic volume (LVEDV). LVEF was calculated by dividing stroke volume (SV) by LVEDV. The simplified Modification of Diet in Renal Disease formula was used to calculate eGFR.

Statistical Analyses

Normality of data was analyzed with the use of the Kolmogorov-Smirnov test combined with Q-Q plots. Normally distributed data are presented as mean ± SD. Nonnormally distributed data are presented as median (interquartile range [IQR]). Categoric variables are presented as percentages. Differences between 2 groups were analyzed with the use of the independent-samples *t* test for normally distributed data, the Mann-Whitney *U* test for nonnormally distributed data, and the Fisher exact test for categoric variables. Univariate linear regression analysis was performed on normally distributed data and log-transformed nonnormally distributed data. Log-transformed HE4 data were used in univariate regression analysis. Results are shown as standardized β coefficient (Sβ), which reflects the change in SD for the dependent variable for 1 SD change in the independent variable, and R^2, also known as the coefficient of determination. Variables with a *P* value of ≤.30 in age-adjusted univariate regression analysis were included in the multivariate linear regression analysis. Forward selection was used to compose a multivariable model. Log-transformed HE4 data were used also in multivariate linear regression analysis. Results are shown as β ± SE and Sβ. The composite end point (all-cause mortality or HF rehospitalization) for HE4 levels below and above median was depicted with the use of Kaplan-Meier curves analyzed with log-rank tests. To calculate the hazard ratios (HRs) and 95% confidence intervals (CIs), the Cox proportional hazards model was used. Assumptions of proportional hazards were tested. Area under the receiver operating characteristics curve (AUC) analysis was used to determine the performance of HE4 as a prognostic biomarker. With the use of a log-rank test for equality of survivors, the expected versus observed event rates for the HE4 median were determined. Statistical analysis was performed with the use of SPSS software (IBM SPSS Statistics version 22; IBM, Armonk, New York). A *P* value of <.05 was considered to indicate statistical significance.

Results

Study Population and Baseline Characteristics

Patients with CHF and control subjects were matched for age and sex (Table 1). In both the control group and the group consisting of patients with HF, frequency distributions of HE4 levels showed nonnormal distributions (Fig. 1A and B), which was confirmed by means of a Kolmogorov-Smirnov test. Median HE4 levels were higher in patients with CHF (69.2 pmol/L, IQR 55.6–93.8) compared with control subjects (56.1 pmol/L, IQR 46.6–69.0; Fig. 1C). Moreover, HE4 levels were higher in patients with higher New York Heart Association (NYHA) functional class (NYHA III: 94.2 pmol/L, IQR 71.1–137.9; NYHA II: 67.3 pmol/L, IQR 54.9–85.5), suggestive of a relation between HE4 levels and HF severity (Fig. 1D). This cohort did not contain NYHA IV patients. All patients with HF and control subjects had serum HE4 levels above the detection limit of 25 pmol/L of the HE4 ELISA.

Baseline characteristics of the total CHF cohort and as stratified below and above the HE4 median are presented in Table 2. All patients with HF were treated with ACE inhibitors or ARBs, and nearly all were treated with β-blockers. Patients with HE4 levels above the median were older and in a higher NYHA functional class. Hypertension and smoking were more prevalent in patients with high HE4 levels. Moreover, NT-proBNP levels and levels of other HF-associated biomarkers, including Gal-3, hsTnT, and ICTP, were higher in the group above the median. Total cholesterol and LDL were, however, lower in the high-HE4 group. Plasma creatinine levels were significantly higher and eGFR lower in patients with high HE4. No difference in HE4 levels in CHF of ischemic etiology (HE4 70.1 pmol/L, IQR 59.5–97.0; n = 73) versus nonischemic etiology (HE4 65.2 pmol/L, IQR 50.6–89.6; n = 28) was observed (*P* = .165).

Factors Associated With HE4

To further investigate which variables were associated, log-transformed HE4 levels were used in linear regression analyses. Both univariate and age-adjusted analyses were performed (Table 3). Increasing age was a strong correlate of HE4 and explained 18.0% of the variance in HE4 levels. In

Table 1. Baseline Characteristics of Patients With Heart Failure and Control Subjects

Characteristic	Control (n = 58)	Heart Failure (n = 101)	*P* Value
Age (y)	63.7 ± 11.0	63.8 ± 10.0	.989
Male sex	54 (93.1%)	94 (93.1%)	1.000
Creatinine (μmol/L)	89.0 ± 25.5	90.0 ± 18.3	.789
HE4 (pmol/L)	56.1 (46.6–69.0)	69.2 (56.6–93.8)	<.001

Data are presented as mean ± SD for normally distributed data, as median (interquartile range) for nonnormally distributed data, and as n (%) for categoric variables. HE4, human epididymis protein 4.

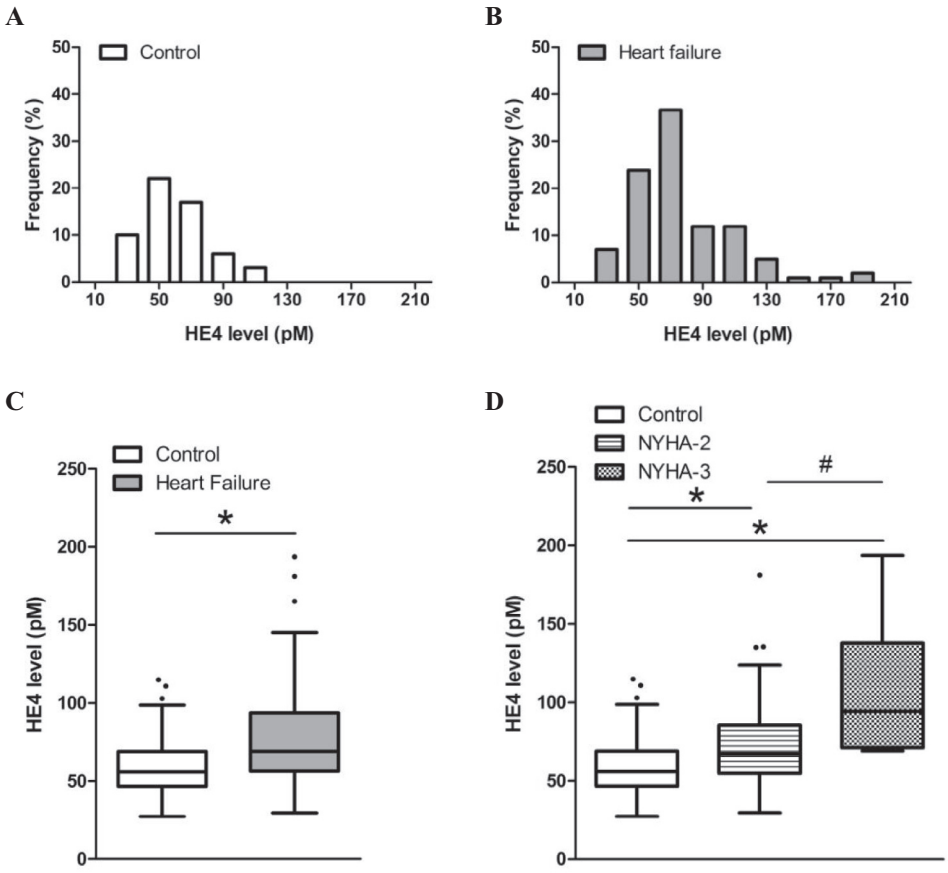

Fig. 1. Serum human epididymis protein 4 (HE4) levels in patients with heart failure (HF) and non-HF control subjects. (**A**) Frequency distribution of HE4 levels in non-HF control subjects from PREVEND (n = 58). (**B**) Frequency distribution of HE4 levels in patients with HF derived from the VitD-CHF cohort (n = 101). Bars indicate percentages within the respective study group. Bin width is 20 pmol/L. Center of the first bin is 10 pmol/L. Center of the last bin is 210 pmol/L. (**C**) HE4 levels in patients with HF derived from the VitD-CHF cohort (n = 101) and non-HF control subjects from PREVEND (n = 58). (**D**) HE4 levels in patients with HF derived from the VitD-CHF cohort with New York Heart Association (NYHA) functional class II (n = 89) and III (n = 12) and non-HF control subjects from PREVEND (n = 58). Bars indicate median. Error bars represent interquartile range. *$P < .05$ vs control group. #$P < .05$ vs NYHA II.

age-adjusted univariate regression analysis, NYHA functional class, HF markers including NT-proBNP and Gal-3, and kidney function markers, among others, remained significantly associated with HE4 levels.

In a stepwise regression selection we identified creatinine, NT-proBNP, Gal-3, hsTnT, and smoking as factors associated with HE4. In this multivariable model, these factors explained 59.5% of the variance in HE4 levels (Table 4). To create a model including HF-related risk factors also, we forced age, ischemic etiology, and LVEF in our multivariable model, which will be referred to as our clinical model (Table 4).

HE4 and HF Outcome

Among 101 patients with CHF, 19 patients (20.9%) died and 13 (15.3%) HF hospitalizations were recorded in a follow-up period of 5 years. The Kaplan-Meier curve for composite end point (all-cause mortality or HF hospitalization) is shown in Fig. 2. In patients with HE4 levels above the median, survival was worse ($P < .001$). To adjust for the time to event, Cox regression analyses were performed and the HRs are presented. Patients with elevated HE4 levels showed a significant HR of 6.3 (CI 2.39–16.5; unadjusted $P < .001$). After adjusting for both the multivariable model and the clinical model (including the multivariable model and HF risk factors), HE4 remained a significant predictor for events (HRs 4.3 [CI 1.50–12.5; $P = .007$] and 3.8 [1.31–11.1; $P = .014$], respectively). Assumptions of proportional hazards were upheld for all tested models.

To determine the performance of HE4 as a prognostic biomarker, AUC analysis was performed. Solitary serum HE4 levels had an AUC of 0.733, which was better than the value of 0.630 for NT-proBNP (Table 5). The AUC of the multivariable model was 0.807. Adding HE4 to this multivariable model resulted in an AUC of 0.840. This was a nonsignificant increase of 3.3% ($P = .245$). With the use of the log-rank test for equality of survivor functions, expected versus observed event rates for the HE4 median were determined and analyzed. More events than expected were observed in the group of patients with HE4 levels above the HE4 median (expected: 12.65 events; observed: 24 events). In the group of patients with HE4 levels below the HE4 median, fewer

Table 2. Baseline Characteristics of VitD-CHF Cohort

Characteristic	Total (n = 101)	Below-Median HE4 (n = 51)	Above-Median HE4 (n = 50)	*P* Value
Age (y)	63.8 ± 10.0	60.8 ± 9.2	66.8 ± 10.0	.002*
Male, n (%)	94 (93.1%)	46 (90%)	48 (96%)	.25
SBP (mm Hg)	116.4 ± 16.9	116.7 ± 14.2	116.0 ± 19.4	.83
DBP (mm Hg)	71.0 ± 10.6	71.1 ± 8.2	70.8 ± 12.6	.87
Hypertension	35 (34.7%)	12 (24%)	23 (46%)	.018*
BMI (kg/m^2)	28.0 ± 4.4	28.2 ± 3.8	27.7 ± 4.9	.60
Diabetes	14 (13.9%)	4 (8%)	10 (20%)	.077
Current smoker	23 (22.8%)	7 (14%)	16 (32%)	.029*
Myocardial infarction	73 (72.3%)	34 (67%)	39 (78%)	.20
Hypercholesterolemia (%)	54 (53.5%)	27 (53%)	27 (54%)	.92
Heart failure history				
NYHA functional class				
II	89 (88.1%)	50 (98%)	39 (78%)	.002*
III	12 (11.9%)	1 (2%)	11 (22%)	
LVEF (%)	34.6 ± 8.2	34.9 ± 7.3	34.4 ± 9.1	.77
Treatment				
ACEi/ARB	101 (100.0%)	51 (100%)	50 (100%)	.21
β-Blocker	98 (97.0%)	50 (98%)	48 (96%)	.55
MRA	29 (28.7%)	11 (22%)	18(36%)	.11
Diuretic	67 (66.3%)	29 (57%)	38 (76%)	.042*
Lipid-lowering drug	78 (77.2%)	38 (75%)	40 (80%)	.51
Laboratory measurements				
eGFR ($mL\ min^{-1}\ 1.73\ m^{-2}$)	80.4 ± 16.5	85.1 ± 14.1	75.6 ± 17.5	.003*
NT-proBNP (pg/mL)	375.5 (201.5–808.3)	311.5 (195.0–515.0)	509.5 (270.0–1462.0)	.002*
Creatinine (μmol/L)	90.0 ± 18.3	84.5 ± 13.1	95.5 ± 21.1	.002*
HbA_{1c} (%)	5.9 (5.7–6.3)	5.9 (5.6–6.1)	6.0 (5.7–6.4)	.093
Total cholesterol (mmol/L)	4.3 (3.7–4.9)	4.5 (3.9–5.2)	3.9 (3.5–4.5)	.012*
LDL (mmol/L)	2.4 (2.0–3.1)	2.6 (2.1–3.3)	2.3 (1.8–2.9)	.005*
Albumin (g/L)	44.0 (43.0–46.0)	45.0 (43.0–47.0)	44.0 (42.0–46.0)	.017*
Urea (mmol/L)	6.3 (5.5–7.5)	6.1 (5.2–6.8)	6.9 (5.9–7.8)	.039*
PRA (nmol/L/h)	5.2 (1.5–19.7)	4.3 (1.5–15.3)	7.0 (1.4–28.7)	.34
Aldosterone (nmol/L)	0.2 (0.1–0.4)	0.2 (0.1–0.3)	0.3 (0.1–0.4)	.036*
FGF-23 (RU/mL)	112.8 (92.5–164.0)	105.0 (85.4–120.5)	136.6 (103.5–184.2)	<.001*
Gal-3 (ng/μL)	16.6 (14.5–19.3)	15.6 (13.4–17.6)	18.4 (15.8–20.4)	<.001*
hsTnT (pg/mL)	4.3 (3.0–11.5)	3.0 (3.0–6.5)	8.4 (3.0–17.2)	<.001*
ICTP (μg/L)	3.4 (2.6–4.3)	3.2 (2.3–3.8)	3.8 (2.8–4.5)	.016*
PIIINP (ug/L)	10.5 (7.0–15.5)	10.0 (7.0–13.0)	11.0 (8.5–19.0)	.10

Data are presented as mean ± SD for normally distributed data, as median (interquartile range) for nonnormally distributed data, and as n (%) for categoric variables. HE4, human epididymis protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; NYHA, New York Heart Assocation; LVEF, left ventricular ejection fraction; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist, eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; HbA_{1c}, glycated hemoglobin; LDL, low-density lipoprotein; PRA, plasma renin activity; FGF-23, fibroblast growth factor 23; Gal-3, galectin-3; hsTnT, high-sensitivity troponin T; ICTP, collagen type 1 cross-linked telopeptides; PIIINP, protocollagen 3 N-terminal propeptide.

*$P < .05$.

events than expected were observed (expected: 16.35 events; observed: 5 events; $\chi^2 = 18.17$; $P < .001$).

Discussion

This study is the first to show that HE4 levels are elevated in patients with CHF. We show that HE4 levels in patients with CHF are correlated with HF risk factors including age, male sex, hypertension, diabetes and HbA_{1c}. Importantly, strong correlations were observed with HF severity, based on NYHA functional class and NT-proBNP levels, and with other HF-associated biomarkers, including Gal-3, FGF-23, hsTnT, and ICTP. Also, an association between HE4 and kidney function was observed. HE4 levels were higher with decreasing renal function. A high HE4 level (above the median) was associated with a higher risk of reaching the composite end point, defined as HF hospitalization or all-cause mortality, in this cohort of patients with CHF.

HE4 and Heart Failure

Previously, we reported that HE4 levels were associated with HF severity and kidney function in patients with AHF.[12] The results of the present study in patients with CHF are in accordance with the data reported in patients with AHF and again showed a strong association with NT-proBNP and Gal-3. Several other studies showed that HE4 is associated with renal diseases,[14,21,22] and a correlation of HE4 and plasma creatinine has been reported.[23] In this study, we limited the patient group to patients with an eGFR >60 $mL\ min^{-1}\ 1.73\ m^{-2}$ to limit the influence of this potential cofounding factor. Despite this selection, a positive correlation with kidney function was still observed. Both NT-proBNP and plasma creatinine levels

Table 3. Relationship Between HE4 Levels and Baseline Characteristics

Characteristic	Univariable Sβ	R^2	*P* Value	Age-Adjusted Sβ	R^2	*P* Value
Age	0.433	0.180	<.001*			
Female sex	–0.255	0.056	.010*	–0.238	0.229	.008*
SBP	–0.031	–0.009	.759	–0.109	0.183	.236
DBP	–0.013	–0.010	.900	–0.006	0.171	.944
Hypertension	0.213	0.036	.033*	0.154	0.195	.093
BMI	–0.027	–0.009	.785	–0.003	0.171	.972
Diabetes	0.217	0.038	.029*	0.253	0.224	.011*
Current smoker	0.178	0.022	.075	0.262	0.239	.004*
Myocardial infarction	0.145	0.011	.148	0.161	0.198	.076
Hypercholesterolemia	–0.057	–0.007	.573	–0.042	0.173	.643
Heart failure history						
NYHA functional class (II vs III)	0.337	0.105	.001*	0.267	0.241	.003*
LVEF	0.008	–0.010	.941	–0.023	0.172	.802
Treatment						
ACEi/ARB	0.000	0.000	1.000	0.000	0.000	1.000
β-Blocker	–0.103	0.001	.307	–0.074	0.177	.418
MRA	0.153	0.014	.126	0.229	0.223	.012*
Diuretics	0.120	0.004	.233	0.117	0.185	.198
Lipid-lowering drug	0.099	0.000	.324	0.066	0.176	.468
Laboratory measurements						
eGFR	–0.431	0.178	<.001*	–0.289	0.236	.005*
NT-proBNP†	0.440	0.185	<.001*	0.314	0.252	<.001*
Creatinine	0.509	0.252	<.001*	0.415	0.330	.001*
HbA_{1c}†	0.220	0.039	.028*	0.194	0.208	.033*
Total cholesterol†	–0.256	0.056	.010*	–0.195	0.208	.034*
LDL†	–0.282	0.070	.004*	–0.227	0.221	.013*
Albumin	–0.234	0.045	.019*	–0.073	0.174	.464
Urea†	0.347	0.112	<.001*	0.199	0.203	.049
PRA†	0.146	0.011	.151	0.212	0.212	.022*
Aldosterone†	0.244	0.050	.014*	0.244	0.233	.007*
FGF-23†	0.422	0.170	<.001*	0.341	0.286	<.001*
Gal-3†	0.466	0.210	<.001*	0.366	0.294	<.001*
hsTnT†	0.520	0.263	<.001*	0.404	0.294	<.001*
ICTP†	0.421	0.168	<.001*	0.316	0.253	.001*
PIIINP†	0.275	0.066	.006*	0.133	0.174	.186

Sβ, standardized beta coefficient, a reflection of the change in dependent variable for 1 SD change in the independent variable. Other abbreviations as in Table 2.
*$P < .05$.
†Log-transformed.

were included as independent explanatory variables of HE4 levels in the multivariable model. Patients with CHF with above-median HE4 levels had a higher risk of reaching the composite end point. Similar results were obtained with HE4 levels above the 95% percentile of the healthy control group (103.3 pmol/L; data not shown). Thus, in patients with CHF, HE4 levels are associated with HF severity and kidney function and are predictive for HF outcome (HF rehospitalization

Table 4. Multivariable Models

Characteristic	β ± SE	Sβ	*P* Value
Multivariable model			
Creatinine	0.549 ± 0.141	0.317	<.001*
Gal-3†	2.678 ± 0.585	0.347	<.001*
hsTnT†	9.943 ± 0.585	0.258	.003*
Current smoker	15.935 ± 5.249	0.215	.003*
NT-proBNP†	5.268 ± 2.489	0.173	.037*
Clinical model			
Creatinine	0.494 ± 0.140	0.328	.001*
Gal-3†	2.945 ± 0.599	0.381	<.001*
hsTnT†	10.154 ± 3.469	0.263	.005*
Current smoker	12.957 ± 5.383	0.175	.019*
NT-proBNP†	7.087 ± 2.596	0.233	.008*
Age	–0.219 ± 0.286	–0.070	.447*
Ischemic etiology	10.903 ± 5.172	0.158	.038*
LVEF	0.536 ± 0.281	0.144	.061

Model including HE4-associated factors: $R^2 = 0.595$. Model including HE4-associated factors and HF risk factors: $R^2 = 0.611$. Abbreviations as in Tables 2 and 3.
*$P < .05$.
†Log-transformed.

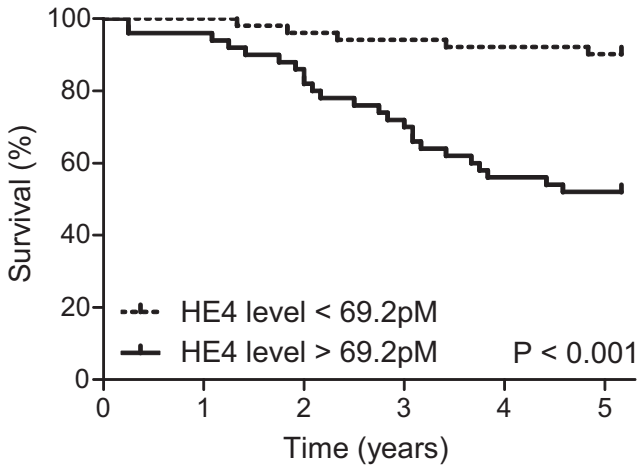

Fig. 2. Kaplan-Meier curves in patients with chronic heart failure and human epididymis protein 4 (HE4) levels above and below the median for the composite end point (heart failure hospitalization or all-cause mortality). $P < .001$.

Table 5. Performance of HE4, HF Biomarkers, and Multivariable Models as Risk Predictors

Predictor	AUC (95% CI)
Biomarkers	
HE4	0.733 (0.644–0.823)
NTproBNP	0.630 (0.526–0.733)
FGF23	0.630 (0.526–0.733)
Gal-3	0.612 (0.507–0.718)
ICTP	0.616 (0.512–0.719)
PIIINP	0.571 (0.463–0.679)
Models	
Multivariable model	0.807 (0.719–0.894)
Multivariable model + HE4	0.840 (0.760–0.920)

AUC, area under the receiver operating characteristic curve; CI, confidence interval; other a bbreviations as in Table 2.

or all-cause mortality). Moreover, from the AUC analysis, we can conclude that solitary serum HE4 levels are a good predictor of HF outcome. The added value of HE4 to the multivariable model, including known HF biomarkers such as NT-proBNP was, however, not significant. This may not be surprising, because these markers in the multivariable model predicted 59.5% of the variance in HE4 level and are thus strongly associated. Much more outcome events may probably be needed to provide adequate statistical power to test whether a new risk marker adds prognostic information to established risk factors in a multivariable model.[24] Nevertheless, 40% of HE4 variance is still unexplained and therefore HE4 levels may provide additional information about a subset of patients and may have additional value for patient stratification. For such reasons, Gal-3 and sST2 biomarkers have been included into the AHA guidelines to potentially provide additional risk stratification,[8] even though for those biomarkers it is not yet clear in what characteristics these patients differ exactly. In our opinion this is a first step that may pave the way for further investigations in HE4 and CHF and may one day help in HF risk stratification and therapeutic treatment. Finally, we note that, compared with our previous AHF study,[12] HE4 levels were lower in the present study with optimally treated CHF patients. We believe this strengthens the statement that HE4 levels are correlated with HF severity. It is tempting to suggest that HE4 levels increase in acute decompensated HF and decline when the situation stabilizes and therefore that HE4 could also be a marker for treatment. This hypothesis should be tested in an AHF study with subsequent follow-up.

HE4 and Fibrosis

Although the exact function of HE4 is still unknown, HE4 sequence shows similarity to proteinase inhibitors, which suggests a functional role of HE4 in fibrosis formation,[13,17] and in the kidney it has been shown that that fibroblast-derived HE4 is a mediator of fibrosis.[18] In a mouse model of renal fibrosis, mice treated with HE4-neutralizing antibodies showed reduced renal fibrosis.[18] In the present study, correlations of HE4 with HF biomarkers of fibrosis were found, of which Gal-3 showed the strongest correlation. Fibrosis and HF development are closely linked, and HE4 has been associated with processes of fibrosis.[18] Therefore, increased levels of HE4 in HF could potentially promote organ fibrosis and/or play a role in fibrosis-induced end-organ damage in HF. Because neutralizing HE4 results in less renal fibrosis in mouse models of renal diseases,[18] we speculate that HE4 could constitute a potential target to reduce fibrosis in HF.

Study Limitations

A limitation of this study is the relatively small sample size, a single-point assessment, and the high percentage of male subjects. Nevertheless, we demonstrate a difference in HE4 levels between patients with HF and control subjects. Control group samples and CHF samples were from 2 different studies, and the control group was composed from the PREVEND study, which consists predominantly of patients with minor microalbuminuria. However, the median HE4 level of the control group was in the same range as previously reported normal values.[14,23]

Conclusion

HE4 levels are increased in patients with CHF and correlate with HF severity, NT-proBNP levels, and renal function. HE4 levels are independently associated with outcome.

Disclosures

None.

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