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The WAP Four-Disulfide Core Domain Protein HE4: A Novel Biomarker for Heart Failure

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Objectives This study investigated clinical determinants and added prognostic value of HE4 as a biomarker not previously described in heart failure (HF).

Background Identification of plasma biomarkers that help to risk stratify HF patients may help to improve treatment.

Methods Plasma HE4 levels were determined in 567 participants of the COACH (Coordinating study evaluating outcomes of Advising and Counseling in Heart failure). Patients had been hospitalized for HF and were followed for 18 months. The primary endpoint of this study was a composite of all-cause mortality and HF hospitalization.

Results HE4 showed a strong correlation with HF severity, according to New York Heart Association functional class and brain natriuretic peptide (BNP) levels ($p < 0.001$). HE4 also showed a positive correlation with GDF15 ($p < 0.001$) and, in addition, correlated with kidney function (estimated glomerular filtration rate [eGFR]; $p < 0.001$). Cox regression analysis revealed that a doubling of HE4 levels was associated with a hazard ratio (HR) of 1.73 (95% confidence interval [CI]: 1.53 to 1.95) for the primary outcome ($p < 0.001$). After correction for age, gender, BNP, and eGFR, the HR was 1.46 (95% CI: 1.23 to 1.72; $p < 0.001$), and after additional adjustment for GDF15, the HR lowered to 1.30 (95% CI: 1.07 to 1.59; $p = 0.009$). The area under the curve in the receiver-operating characteristic curve analysis increased from 0.727 to 0.752 when HE4 was included in the clinical evaluation ($p = 0.051$). The integrated discrimination improvement and net reclassification index for reclassification showed significant improvements when HE4 was added to the clinical model, and this remained significant after BNP inclusion in the model.

Conclusions HE4 plasma levels are correlated with markers of HF severity, show prognostic value, and can improve risk assessment in HF. (J Am Coll Cardiol HF 2013;1:164–9) © 2013 by the American College of Cardiology Foundation

Heart failure (HF) is the final common syndrome of most cardiovascular diseases, including myocardial infarction, hypertension, valvular disease, cardiomyopathy, and others. Once HF ensues, it is associated with high morbidity and mortality, especially because HF often is diagnosed after it has already progressed (1–3). The number of HF patients is estimated to be between 1% and 2% of the total population (2) and is expected to increase dramatically in the next decade because of the ageing population.

There is not a single diagnostic or prognostic test for HF, reflecting the heterogeneous background of HF. Prognosis is estimated using several key patient characteristics such as age, comorbidity, and severity of disease (New York Heart Association [NYHA] class, left ventricular ejection fraction [LVEF]). Natriuretic peptides have clearly enhanced management of patients with HF, and current guidelines mention brain natriuretic peptide (BNP), its precursor N-terminal pro-brain natriuretic peptide (NT-prBNP), and N-terminal pro-atrial natriuretic peptide (NT-proANP) as diagnostic biomarkers (2,3). With the increasing availability of therapeutic strategies and novel treatment modalities, decision making in the care of the patient, however, has become more difficult. The demand for patient-tailored therapeutic strategies requires a careful risk stratification of patients with HF and requires identification of new biomarkers that may fulfill these purposes.

A number of potential new biomarkers for HF have recently been described, including galectin-3, ST2, and GDF15 (4–7), but none has come into standard clinical

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use so far. This underscores the fact that despite impressive technical developments in genomics and proteomics, identification of useful biomarkers is still a daunting task. Plasma HE4 (also termed WFDC-2) is currently in use for monitoring recurrence of progression of epithelial ovarian cancer (8), but its biomarker potential has not been investigated in other diseases. During standard specificity testing, a strong correlation was observed between HE4 and severity of heart failure (NYHA functional class) (Alere Company, unpublished results, March 2012), which prompted the present investigation.

Here we aimed to evaluate whether or not HE4 could constitute a potential new HF biomarker. Plasma samples of the COACH (Coordinating study evaluating Outcomes of Advising and Counseling in Heart failure) trial were analyzed.

Methods

Study design and outcome parameters. This is a substudy of the COACH trial. The design and outcomes of the COACH trial (NCT 98675639) have been published (9,10). Plasma (for determination of HE4 and of other biomarkers) was available from 567 patients during the index admission, and these patients formed the cohort for the present substudy. This study complies with the Declaration of Helsinki and local medical ethics committees approval, and all patients provided written informed consent. Detailed information and further methods can be found in the Online Methods section.

Statistical analysis. HE4 levels (ng/ml) were divided into quartiles (first quartile 0.7 to 3.5; second quartile 3.5 to 5.6; third quartile 5.6 to 10.1; fourth quartile 10.1 to 63.3). Baseline demographic values are mean ± SD or median (interquartile ranges [IQR]) when variables were non-normally distributed. Detailed statistical analysis is described in the Online Methods section.

Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
ARB = angiotensin receptor blockers
AUC = area under the curve
BNP = brain natriuretic peptide
CRP = C-reactive protein
eGFR = estimated glomerular filtration rate
HF = heart failure
HR = hazard ratio
IDI = integrated discrimination improvement
IQR = interquartile range
LVEF = left ventricular ejection fraction
NRI = net reclassification index
NYHA = New York Heart Association
WFDC-2 = WAP four-disulfide core domain protein 2

Results

Study population. Baseline characteristics of the 567 patients in this subanalysis (Table 1) were comparable to those of the total COACH cohort (n = 1,023; data not shown) (10). Mean age of the study population was 71 ± 11 years, and 62% of patients were male. Approximately one-half of the patients were NYHA class II, and the other half was class III, and 3% were class IV. LVEF was measured predominantly by echocardiography, and the mean LVEF was 33 ± 14%. Mean eGFR was 54 ml/min/1.73 m^2, median BNP value was 456

Table 1 Baseline Parameters According to Plasma HE4 Levels

Variable	Quartiles of HE4 (ng/ml) Quartile 1 (0.7–3.5)	Quartile 2 (3.5–5.6)	Quartile 3 (5.6–10.1)	Quartile 4 (10.1–63.3)	Overall Sample	p Value
N	142	141	142	142	567	
Age (yrs)	66 ± 12	70 ± 11	74 ± 9	75 ± 9	71 ± 11	<0.001
Gender (% male)	56	60	63	69	62	0.13
NYHA functional class II/III/IV (%)	66/32/2	51/48/1	40/54/6	30/65/5	47/50/3*	<0.001
BMI (kg/m^2)	28 ± 5	27 ± 5	27 ± 6	26 ± 5	27 ± 5†	0.050
LVEF (%)	33 ± 14	33 ± 16	32 ± 13	34 ± 13	33 ± 14‡	0.82
LVEF ≥40%	35	31	28	30	31‡	0.70
LVEF ≥55%	10	13	9	11	11‡	0.75
Medical history (%)						
Hypertension	39	39	44	47	42	0.46
Myocardial infarction	33	41	41	49	41	0.070
Diabetes	25	28	31	38	30	0.093
Atrial fibrillation	35	42	52	55	46	0.002
COPD	23	21	32	36	28	0.018
CVA	8	9	11	13	10	0.56
Medication (%)						
ACE inhibitors + ARB	87	87	82	73	82	0.005
Beta-blocker	73	72	53	69	67	0.001
Diuretics	93	95	98	96	96	0.21

Values are n, mean ± SD, or %. *N = 564, †N = 539, ‡N = 515.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blockers; BMI = body mass index; COPD = chronic obstructive pulmonary disease; CVA = cardiovascular accident; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

pg/ml, and patients were taking standard medications for HF, including angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARB), beta-blockers, and diuretics.

Identification of HE4 and baseline characteristics of patients stratified to HE4 levels. Using bead-based multiplex immunoassay screening platform, we identified HE4 as a protein whose plasma levels showed a strong correlation with HF severity. In Table 1, the baseline characteristics of patients are shown, according to quartiles of plasma HE4 levels. Patients with higher HE4 levels clearly had an unfavorable clinical profile: they were older, were in higher NYHA class (both $p < 0.001$), and had more comorbidities, including atrial fibrillation and chronic obstructive pulmonary disease. ACE inhibitors, ARBs, and beta-blocker treatments were less often prescribed in patients in the higher HE4 quartiles ($p = 0.005$ and $p = 0.001$, respectively).

Relation between HE4 and other HF plasma markers and kidney function. Using Spearman's rank correlation coefficient, we observed a weak but significant positive correlation among BNP, NT-proBNP, and plasma HE4 levels (Table 2). The positive correlation was stronger with galectin-3, a fibrosis biomarker with predictive value in HF patients (11). The strongest association was observed with GDF15, an emerging prognostic biomarker in patients with cardiovascular disease and HF (6). The association between HE4 levels and the inflammation biomarker C-reactive protein (CRP) was weak. We also observed significant correlations between kidney function indexes, including blood urea nitrogen, serum creatinine, and estimated glomerular filtration rate (eGFR).

Association between baseline HE4 and primary outcome. In 18 months, 240 patients reached the primary outcome (92 deaths and 148 rehospitalizations due to worsening HF). Kaplan-Meier curves show that the risk for the primary outcome was clearly increased for patients in the higher quartiles of HE4, especially quartile 4 (Fig. 1A) ($p < 0.001$, log-rank test). For the fourth quartile, this appeared more pronounced compared to BNP (Fig. 1B). The crude and adjusted associations between the log2 transformed HE4 values and the primary outcome are shown in Table 3. The crude hazard ratio (HR) for the risk of experiencing the primary outcome was 1.73 per doubling of HE4. Adjustments for age and gender only marginally lowered this HR to 1.67. Additional adjustment for BNP further mitigated the HR of HE4 to 1.58, and a further decrease to 1.46 was observed after adjusting for eGFR and to 1.30 after adjustment for GDF15. Also for the separate endpoints, admission for HF and death, significant ($p < 0.001$) changes in HRs of 1.53 and 1.93, respectively, were found (Online Table 1 and 2). These HRs were still significant after correction for gender, age, and BNP.

Performance of HE4 as a prognostic biomarker. As shown in Table 4, prediction based on the clinical model resulted in an area under the curve (AUC) of 0.7272.

Table 2 Correlation and Concentrations of Plasma Markers in Relation to Plasma HE4

Variable	Quartiles of HE4 (ng/ml)				Overall Sample	Spearman Correlation Coefficient	Spearman p Value
	Quartile 1 (0.7–3.5)	Quartile 2 (3.5–5.6)	Quartile 3 (5.6–10.1)	Quartile 4 (10.1–63.3)			
N	142	141	142	142	567		
eGFR	69 ± 17	61 ± 17	48 ± 16	38 ± 15	54 ± 20	−0.634 (N = 557)	<0.001
BUN	8.2 (6.8–10.4)	9.7 (7.5–12.3)	12.8 (9.7–16.2)	17.6 (13.1–24.0)	11.1 (8.3–15.6)	0.602 (N = 509)	<0.001
Creatinine	94 ± 24	107 ± 27	132 ± 38	175 ± 72	127 ± 54	0.640 (N = 557)	<0.001
NGAL	105 (76–160)	101 (73–131)	129 (98–175)	156 (104–197)	119 (86–170)	0.253 (N = 567)	<0.001
Sodium	140 ± 4	139 ± 4	138 ± 5	139 ± 5	139 ± 4	−0.099 (N = 559)	0.020
Potassium	4.1 ± 0.5	4.2 ± 0.5	4.3 ± 0.5	4.4 ± 0.6	4.2 ± 0.5	0.153 (N = 558)	<0.001
Hb	8.6 ± 1.4	8.4 ± 1.1	7.9 ± 1.1	7.8 ± 1.2	8.1 ± 1.2	−0.275 (N = 312)	<0.001
HCT	0.41 ± 0.06	0.41 ± 0.06	0.39 ± 0.06	0.39 ± 0.06	0.40 ± 0.06	−0.170 (N = 261)	0.006
BNP	303 (149–540)	451 (210–906)	477 (207–904)	634 (337–1435)	456 (203–903)	0.270 (N = 544)	<0.001
NT-proBNP	1,655 (760–2,688)	2,253 (1,407–4,062)	3,159 (1,347–5,474)	6,163 (2,483–14,070)	2,532 (1,310–5,678)	0.418 (N = 538)	<0.001
GDF15	1.7 (1.3–2.2)	2.5 (1.9–3.2)	3.2 (2.4–4.2)	5.1 (3.7–6.4)	2.8 (1.9–4.3)	0.728 (N = 567)	<0.001
CRP	10.4 (4.2–33.0)	9.6 (4.2–21.5)	14.4 (5.6–33.0)	15.1 (5.4–33.0)	11.4 (4.7–33.0)	0.102 (N = 567)	0.015
GAL3	16 ± 6	19 ± 6	24 ± 9	30 ± 12	22 ± 10	0.570 (N = 565)	<0.001

Values are n, mean ± SD, or median (interquartile range).
BNP = brain natriuretic peptide (pg/ml); BUN = blood urea nitrogen (mg/dl); Creatinine = mmol/l; CRP = C-reactive protein (μg/ml); eGFR = estimated glomerular filtration rate (ml/min/1.73 m^2); GAL3 = galectin-3 (ng/ml); GDF15 = growth differentiation factor 15 (ng/ml); Hb = hemoglobin (g/dl); HCT = hematocrit; IQR = interquartile range; NGAL = neutrophil gelatinase-associated lipocalin (ng/ml); NT-proBNP = N-terminal-proBNP (pg/ml); Potassium = mmol/l; Sodium = mmol/l.

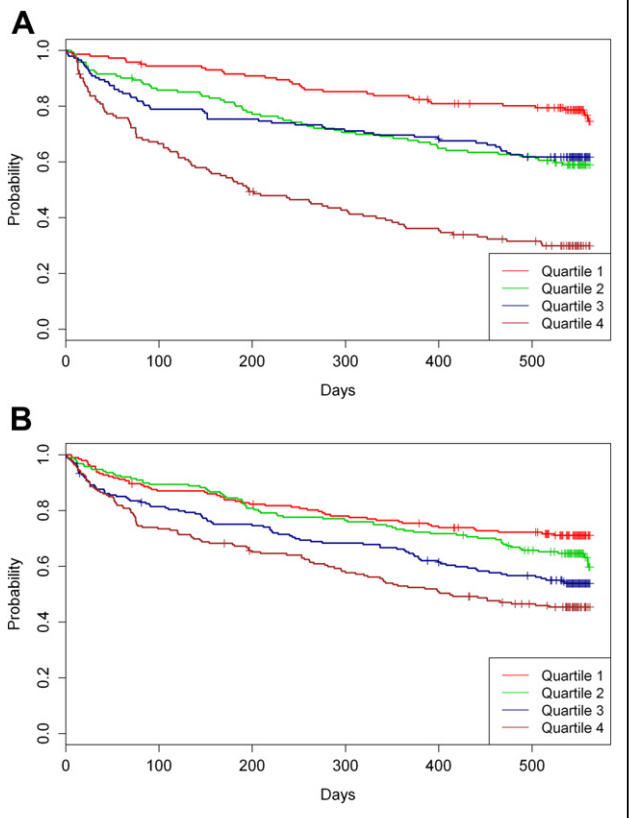

Figure 1 **Primary Outcome**

Kaplan-Meier estimates for HE4 quartiles **(A)** and for brain natriuretic peptide quartiles **(B)**.

Addition of HE4 increased the AUC to 0.7515 ($p = 0.051$), whereas the addition of BNP increased this value to 0.77594 ($p = 0.013$). The integrated discrimination improvement (IDI) and net reclassification index (NRI) values were calculated and are also shown in Table 4. These revealed a significant improvement when HE4 was added to the clinical model. The improvement was comparable to the improvement that is achieved when BNP is added to the clinical model. CRP levels, another suggested noncardiac specific prognostic marker for HF, did not significantly improve prognosis in our clinical model (Online Table 3). Adding HE4 to a model including clinical predictors and BNP showed a further significant improvement in IDI and NRI (Table 4). Similar results were obtained for the single endpoint of death, but no significance was obtained for the single endpoint of HF-related readmission when BNP was included (Online Table 4 and 5). Finally, we added HE4 to a model containing all the demographic, clinical, and biological variables included in our previously published prognostic model (12). This addition still resulted in a significant IDI value, but the NRI was no longer significant.

Table 3 **Primary Outcome Prediction Using Cox Regression Analysis: Death or Admission for Heart Failure: Doubling of HE4, BNP, and GDF15**

Variable	Hazard Ratio (95% CI)	p Value
HE4 (doubling)	1.73 (1.53–1.95)	<0.001
Adjusted for age, gender	1.67 (1.47–1.89)	<0.001
Adjusted for age, gender, BNP (doubling)	1.58 (1.38–1.81)	<0.001
Adjusted for age, gender, BNP (doubling), eGFR	1.46 (1.23–1.72)	<0.001
Adjusted for age, gender, BNP (doubling), eGFR, GDF15 (doubling)	1.30 (1.07–1.59)	0.009

BNP = brain natriuretic peptide; CI = confidence interval.

Discussion

This is the first study to describe HE4 as a novel biomarker for HF. We showed that HE4 was strongly associated with HF severity and outcome and that this association was independent of other established risk factors for poor outcome in HF, including age, gender, BNP level, and renal function. Moreover, addition of HE4 to commonly used clinical parameters resulted in improved reclassification as assessed by NRI and IDI. Also, after we added the gold standard biomarker BNP to the clinical model, HE4 improved reclassification. Finally, after accounting for all demographic, clinical, and biological variables included in our previously published prognostic model (12), addition of HE4 still resulted in a significant value of IDI. The value of the NRI, however, was no longer significant.

HE4 function. HE4 (also termed WFDC2) was originally identified as a major human epididymis-specific protein with secretory properties (13). Later studies, however, showed strong expression of the gene in the respiratory tract, nasopharynx, and salivary glands; and moderate expression in kidney and low expression in other organs (14–16). Thus, our observed association between serum HE4 levels and HF severity does not necessarily indicate a direct function of HE4 in the heart itself but may rather be an indirect response of other tissues that become affected by the HF syndrome.

HE4 belongs to the class of whey four disulfide core domain (WFDC) proteins. The two best studied proteins of this family, SLPI and elafin, were originally identified on the basis of their antiproteinase activity (17,18), but it is questionable whether HE4 has any antiproteinase activity (19). Other functions linked to WFDC include antimicrobial and immunomodulatory properties (19,20). Our observation that HE4 serum levels show association with GDF15 levels may further hint at a role for this protein in the immune system. GDF15 acts as an inhibitor of polymorphonuclear leukocyte infiltration and leukocyte infiltration in the heart in a myocardial infarct model (21). In HF patients, inflammatory activity is increased (22), and it is tempting to suggest that like GDF15, HE4 may have an immunomodulatory function.

JACC: Heart Failure Vol. 1, No. 2, 2013
April 2013:164–9

Table 4 Performance Metrics of HE4 in Risk Prediction Models

Model	AUC (95% CI)	IDI (95% CI)	Continuous NRI (95% CI)
Clinical model*	0.7272 (0.6819 to 0.7725)	NA	NA
Clinical model + HE4 (doubling)†	0.7515 (0.7082 to 0.7948) p = 0.0509	0.0368 (0.0190 to 0.0546) p < 0.0010	0.4537 (0.2442 to 0.6405) p < 0.0010
Clinical model + BNP (doubling)†	0.7594 (0.7166 to 0.8021) p = 0.0126	0.0383 (0.0213 to 0.0552) p < 0.0010	0.4273 (0.2053 to 0.6308) p < 0.0010
Clinical model + BNP (doubling) + HE4 (doubling)‡	0.7699 (0.7280 to 0.8118) p = 0.2037	0.0205 (0.0071 to 0.339) p = 0.0027	0.3102 (0.1170 to 0.5241) p = 0.0031
Clinical model + BNP (doubling) + eGFR+ sodium‡	0.7650 (0.7226 to 0.8075) p = 0.1962	0.0228 (0.0087 to 0.0369) p = 0.0015	0.2364 (0.0206 to 0.4606) p = 0.0350
Clinical model + BNP (doubling) + eGFR + sodium + HE4 (doubling)§	0.7702 (0.7283 to 0.8121) p = 0.3715	0.0100 (0.0008 to 0.0191) p = 0.0323	0.1509 (−0.0328 to 0.3840) p = 0.1547

The three cut-off point categories selected were 0% to 10%, 10% to 20%, and 20% to 100%. *Clinical model includes age, sex, diastolic blood pressure, systolic blood pressure, stroke, myocardial infarction, atrial fibrillation, peripheral arterial disease, diabetes, left ventricular ejection fraction, previous heart failure hospitalization. †In comparison to the clinical model. ‡In comparison to the clinical model + BNP (doubling). §In comparison to the clinical model + BNP (doubling) + eGFR + sodium.

AUC = area under the curve; BNP = brain natriuretic peptide; eGFR = estimated glomerular filtration rate; IDI = integrated discrimination improvement; NA = not applicable; NRI = net reclassification index

HE4 as a prognostic biomarker. HE4 was originally identified as a biomarker for ovarian carcinoma and was reported to be less frequently positive in nonmalignant disease than CA125, a clinically accepted ovarian cancer marker (23), and this was confirmed by others (24,25). The U.S. Food and Drug Administration have approved HE4 for monitoring recurrence of progression of epithelial ovarian cancer (8). The decision to measure HE4 in COACH trial samples was made because of preliminary findings observed during cancer marker specificity experiments that showed a strong association between elevated HE4 levels and NYHA class in a set of HF compared to normal samples (Alere Company, unpublished data, March 2012). It is clear that HE4 is not a disease-specific biomarker, and it is unlikely to be useful for large-scale diagnostic screening for a specific disease in the general population. For prognostic purposes, monitoring disease progression or monitoring response to therapy specificity may be less important, and hence, HE4 may be well suited for these purposes. This is in line with its use for monitoring recurrence of ovarian cancer. The overall expectation of a cardiovascular biomarker is to enhance the clinician's ability to optimally treat the patient (26), and our data show that HE4 levels are strongly associated with HF severity and outcome and improve classification of HF patients. Whether HE4 also has value in monitoring response to HF therapy requires future investigations.

Study limitations. Although the clinical characteristics of this subset did not differ from those of the entire COACH cohort, we could only measure plasma HE4 levels in a subset of patients for whom baseline plasma samples were available. Sampling was performed at the time of discharge and so occurred at variable time points and at different levels of recompensation. The COACH trial included Dutch (Caucasian) patients, so results may not be generalizable to other HF patients. We analyzed HE4 levels only in one patient cohort, and it will be important to replicate these findings in the future in other HF cohorts.

Conclusions

Herein we showed that HE4 is associated with HF severity and outcome and identified HE4 as a strong and independent prognostic biomarker for HF outcome. HE4 is not a tissue-specific biomarker, and several other parameters and biomarkers are strongly correlated with HE4, giving potential insight into pathophysiological pathways involved. HE4 improves risk classification and hence could potentially improve disease management.

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Key Words: biomarker ■ HE4 ■ heart failure ■ prognostic marker ■ WFDC-2.

APPENDIX

For an expanded Methods section and supplemental tables, please see the online version of this article.