JACC: HEART FAILURE
VOL. 5, NO. 10, 2017

ISSN 2213-1779/$36.00
PUBLISHED BY ELSEVIER
http://dx.doi.org/10.1016/j.jchf.2017.07.013

CLINICAL RESEARCH

Utility of Growth Differentiation Factor-15, A Marker of Oxidative Stress and Inflammation, in Chronic Heart Failure

Insights From the HF-ACTION Study

Abhinav Sharma, MD,[a] Susanna R. Stevens, MS,[a] Joseph Lucas, PhD,[a] Mona Fiuzat, PhD,[a] Kirkwood F. Adams, MD,[b] David J. Whellan, MD,[c] Mark P. Donahue, MD, MHS,[d] Dalane W. Kitzman, MD,[e] Ileana L. Piña, MD,[f] Faiez Zannad, MD, PhD,[g] William E. Kraus, MD,[d] Christopher M. O'Connor, MD,[h] G. Michael Felker, MD, MHS[a]

ABSTRACT

OBJECTIVES This study sought to determine the relationship between growth differentiation factor (GDF)-15 and clinical outcomes in ambulatory patients with heart failure and reduced ejection fraction (HFrEF).

BACKGROUND The prognostic utility of GDF-15, a member of the transforming growth factor-β cytokine family, among patients with HF is unclear.

METHODS We assessed GDF-15 levels in 910 patients enrolled in the HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training) trial, a randomized clinical trial of exercise training in patients with HFrEF. Median follow-up was 30 months. Cox proportional hazard models assessed the relationships between GDF-15 and clinical outcomes.

RESULTS The median GDF-15 concentration was 1,596 pg/ml. Patients in the highest tertile of GDF-15 were older and had measurements of more severe HF (higher N-terminal pro–B-type natriuretic peptide [NT-proBNP] concentrations and lower peak oxygen uptake on cardiopulmonary exercise testing [CPX]). GDF-15 therapy was a significant predictor of all-cause death (unadjusted hazard ratio [HR]: 2.03 when GDF-15 was doubled; $p < 0.0001$). This association persisted after adjustment for demographic and clinical and biomarkers including high sensitivity troponin T (hs-TnT) and NT-proBNP (HR: 1.30 per doubling of GDF-15; $p = 0.029$). GDF-15 did not improve discrimination (as measured by changes in c-statistics and the integrated discrimination improvement) in addition to baseline variables, including hs-TnT and NT-proBNP or variables found in CPX testing.

CONCLUSIONS In demographically diverse, well-managed patients with HFrEF, GDF-15 therapy provided independent prognostic information in addition to established predictors of outcomes. These data support a possible role for GDF-15 in the risk stratification of patients with chronic HFrEF. (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training [HF-ACTION]; NCT00047437) (J Am Coll Cardiol HF 2017;5:724–34)

From the [a]Duke Clinical Research Institute, Durham, North Carolina; [b]Division of Cardiology, University of North Carolina Chapel Hill, Chapel Hill, North Carolina; [c]Division of Cardiology, Thomas Jefferson University, Philadelphia, Pennsylvania; [d]Division of Cardiology, Duke University School of Medicine, Durham, North Carolina; [e]Division of Cardiology, Wake Forest School of Medicine, Winston-Salem, North Carolina; [f]Division of Cardiology, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, New York; [g]INSERM, CIC 1433, Université de Lorraine, CHRU, Nancy, France; and the [h]Inova Heart and Vascular Institute, Fairfax, Virginia. Dr. Sharma has received funding from the Alberta Innovates Health Solution Clinician Scientist fellowship, Roche Diagnostics, and a Bayer-Vascular Canadian Cardiovascular Society research grant. Dr. Fiuzat has received research funding

Chronic heart failure (HF) affects more than 5 million people in the United States and is one of the most common causes of rehospitalization (1-3). The overall prognosis remains poor, with 5-year mortality exceeding 50%, despite advances in therapy (2,4,5). As the number of invasive and noninvasive HF therapies increase, risk stratification and prognostication become essential to identify patients who would most benefit from these treatments. Currently the U.S. Food and Drug Administration has approved 4 biomarkers to aid in the prognostication for HF patients: B-type natriuretic peptide (BNP), N-terminal pro-B-type natriuretic peptide (NT-proBNP), galectin-3, and ST-2. Current HF guidelines indicate that measurements of other clinically available tests such as natriuretic peptides (NT-proBNP and BNP) and markers of myocardial injury (cardiac troponin T or I) may be considered for risk stratification (5). Although a variety of other potential prognostic biomarkers have been identified in HF, their clinical value remains uncertain.

Growth differentiation factor (GDF)-15, a member of the transforming growth factor-β family, is secreted from a range of cells such as adipocytes and myocytes in response to cellular ischemia, mechanical strain, and oxidative stress (6-9). Prior studies have suggested that GDF-15 provides prognostic utility across a spectrum of cardiovascular diseases in addition to existing clinical risk factors and biomarkers (10-15). However, robust data for GDF-15 in well-treated patients with chronic HF are limited, especially in the context of other guideline-recommended HF biomarkers such as natriuretic peptides and high-sensitivity troponin. A prior analysis from the ValHeFT (Valsartan Heart Failure Trial) (16) identified the fact that GDF-15 was independently associated with mortality; however, there was low use of evidence-based medical therapy and a demographically homogenous patient population. An evaluation of the prognostic role of GDF-15 in a cohort of patients more reflective of current HF practice was needed. Furthermore, there was a paucity of data for the association between GDF-15 and other critical measurements of HF status, such as exercise capacity. In the current study, we evaluated, first, the association between GDF-15 and other biological covariates; second, the association between GDF-15 functional status and exercise capacity; and third, the relationship between GDF-15 and clinical outcomes in a well-characterized cohort of ambulatory patients with HF with reduced ejection fraction (HFrEF) enrolled in the HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training) trial.

ABBREVIATIONS AND ACRONYMS

BNP = B-type natriuretic peptide

GDF = growth differentiation factor

HF = heart failure

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

METHODS

The design, rationale, and primary results of the HF-ACTION (NCT00047437) trial have been previously reported (17,18). Briefly, HF-ACTION was a randomized clinical trial funded by National Heart, Lung, and Blood Institute, evaluating the effects of exercise training in addition to usual care versus usual care alone on long-term morbidity and mortality in patients with symptomatic chronic HF and left ventricular systolic dysfunction (New York Heart Association [NYHA] functional classes II to IV and left ventricular ejection fraction [LVEF] of <35%). Patients were receiving guideline-based HF therapy prior to randomization. The study enrolled 2,331 patients and had a primary composite endpoint of time to all-cause death or all-cause hospitalization. Patients had a median follow-up of 30 months. All deaths and first cardiovascular hospitalizations were adjudicated by a blinded, independent clinical event committee. HF-ACTION was approved by local institutional review boards, and all enrolled patients provided written informed consent.

BIOMARKER ASSESSMENT. A subset of patients enrolled in the HF-ACTION study agreed to participate in the biomarker substudy. Blood samples were obtained on the same day as baseline cardiopulmonary exercise (CPX) testing was conducted but before exercise. Samples were collected from the peripheral vein into EDTA-containing tubes, centrifuged immediately, and stored at −70°C for subsequent analysis.

GDF-15 concentrations were measured in a core laboratory from baseline samples (n = 910), using sensitive sandwich-immunoassay monoclonal

from Roche. Dr. Adams has received research funding from and is a consultant for Roche. Dr. Zannad has received personal fees from Roche Diagnostics, Janssen, Bayer, Pfizer, Novartis, Boston Scientific, Resmed, Amgen, CVRx, Quantum Genomics, Takeda, General Electric, Boehringer Ingelheim, Relypsa, ZS Pharma, AstraZeneca, GlaxoSmithKline, Cardiorenal, and Cardiovascular clinical trials. Dr. O'Connor has received research funding from Roche. Dr. Felker has received research funding from Roche. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. John R. Teerlink, MD, served as Guest Editor for this paper.

Manuscript received March 7, 2017; revised manuscript received July 3, 2017, accepted July 27, 2017.

antibodies (Elecsys GDF-15 assay, Roche Diagnostics, Indianapolis, Indiana). Details of this assay have been previously described (19). The assay has a lower limit of detection of 400 pg/ml, an upper limit of detection of 20,000 pg/ml, an intra-assay coefficient of variation of ≤3.0%, and an interassay coefficient of variation of ≤4.6%. The core laboratory was blinded to all clinical data.

CLINICAL OUTCOMES. The primary clinical outcome of interest for the current analysis was the relationship between GDF-15 concentrations and all-cause mortality. The secondary outcomes of interest were, first, the composite of all-cause death or all-cause hospitalization (HF-ACTION primary endpoint), and second, the composite of cardiovascular death or HF hospitalization. We also assessed the relationship between baseline GDF-15 and baseline assessments of functional capacity including NYHA functional class, 6-min walk distance, and maximal oxygen consumption (peak Vo_2) by cardiopulmonary exercise testing (CPX).

STATISTICAL ANALYSIS. Baseline characteristics are presented as medians (25th, 75th percentiles) and numbers (percentages). The associations between GDF-15 tertile and baseline characteristics were compared using the Kruskal-Wallis test for continuous and ordinal variables and the chi-square test for categorical variables. Event rates by tertile of GDF-15 are shown with Kaplan-Meier curves. To assess the relationship between GDF-15 and baseline clinical and biomarker variables, a model was developed using linear regression with backward elimination and alpha of <0.10 for retention. Modeling for time-to-event variables were done with complete case analysis. There were 716 patients with complete data for the all-cause death model, 646 for the all-cause death or hospitalization model, and 640 for cardiovascular death or HF hospitalization model. GDF-15 was a continuous variable in all models but was log transformed for analysis because it was not normally distributed. For the clinical outcomes of interest, Cox proportional hazards models were used. Hazard ratios (HRs) were calculated for the $\log_2$ such that the HR represented the risk per 2-fold greater value of GDF-15; in place of to create a clinically more interpretable reference for the increase in GDF-15.

Adjustment variables included demographics (age, sex, and race) and a comprehensive set of predictors that had previously been identified in the HF-ACTION cohort for each endpoint (1,20) (Online Table 1a). Because CPX testing is not routinely available in some clinical settings, the adjusted models were examined with and without CPX variables. The baseline CPX variables used in the adjustment models included peak Vo_2 by Weber class, ventricular conduction abnormality, minute ventilation/carbon dioxide production, and exercise duration (Online Table 1b). Models were repeated with the inclusion of GDF-15 × randomized treatment interaction term to assess for evidence of a differential treatment response to the study intervention based on GDF-15 concentrations. Model discrimination and risk predictions with and without GDF-15 were evaluated by using the c-index, continuous net reclassification index (NRI), and integrated discrimination improvement (IDI). Individual biomarkers were also added to the clinical model separately, and the measurements of discrimination were compared. The proportional hazards assumption was checked for each endpoint in the full models (including demographic, clinical, CPX, and biomarker data), and no deviations were identified. For each model comparison, the summary measurements were reported along with 95% bootstrap confidence intervals (CIs) based on 999 replications.

Data were analyzed using SAS version 9.4 software (SAS, Cary, North Carolina). Statistical significance was based on a p value of ≤0.05.

RESULTS

BASELINE CHARACTERISTICS. Baseline characteristics for the HF-ACTION study population are shown in **Table 1**. The biomarker substudy cohort was broadly similar to the overall HF-ACTION population (data not shown). Generally, the study population was similar to that from other chronic HFrEF clinical trials, with the exception of a high representation of nonwhite patients (34%) and women (29%) in the current study. Overall, the patients were medically well managed: 95% were receiving beta blockers; 94% were receiving angiotensin-converting enzyme inhibitors or angiotensin receptor blockers; and 45% were receiving aldosterone antagonists.

GDF-15 tertiles were identified as follows: lowest tertile was <1,173 pg/ml; middle tertile was 1,173 to 2,252 pg/ml; and the highest tertile was >2,252 pg/ml. Compared to patients in the lowest tertile of GDF-15 values, patients in the highest tertile of GDF-15 were older and had more markers of severe HF, including higher prevalence of comorbidities, higher NYHA functional class, higher NT-proBNP, and higher high sensitivity troponin T (hs-TnT). Use of evidence-based medical therapies tended to be lower in patients with higher GDF-15 levels, and loop diuretic doses tended to be

TABLE 1 Baseline Patient Characteristics by Tertile of GDF-15

	Total HF-ACTION Population (N = 2,331)	Lowest GDF-15 Tertile <1,173 pg/ml (n = 303)	Intermediate GDF-15 Tertile 1,173–2,252 pg/ml (n = 304)	High GDF-15 Tertile >2,252 pg/ml (n = 303)	p Value
Age, yrs	59.3 (51.1–68.0)	50.5 (41.0–57.7)	60.9 (54.3–68.4)	66.5 (58.1–75.6)	<0.0001
Females	661 (28.4)	127 (41.9)	75 (24.7)	61 (20.1)	<0.0001
Whites	1,426 (62.1)	150 (50.5)	211 (69.9)	215 (71.9)	<0.0001
Ischemic cause	1,197 (51.4)	81 (26.7)	175 (57.6)	204 (67.3)	<0.0001
HF hospitalization in prior 6 months	610 (26.4)	85 (28.3)	68 (22.5)	90 (29.9)	0.098
LVEF, %	24.7 (20.0–30.1)	25.2 (20.0–30.1)	23.9 (18.8–30.1)	24.3 (19.8–29.6)	0.38
Systolic blood pressure, mm Hg	111 (100–126)	116 (102–126)	112 (103–127)	110 (100–127)	0.30
Heart rate, beats/min	70 (63–77)	72 (64–80)	70 (64–78)	70 (64–79)	0.56
CPX duration, min	9.6 (6.9–12.0)	11.0 (8.7–13.7)	10.0 (7.9–11.6)	7.5 (5.5–10.0)	<0.0001
Peak Vo_2, ml/kg/min (CPX test)	14.4 (11.5–17.7)	16.5 (13.5–19.6)	14.9 (12.4–17.5)	12.3 (9.8–14.7)	<0.0001
VE/Vco_2 slope	32.6 (28.1–38.5)	29.6 (25.7–33.9)	32.1 (28.3–37.0)	36.7 (31.6–44.0)	<0.0001
6-min walk distance, m	371 (299–435)	404 (331–466)	378 (314–439)	324 (258–388)	<0.0001
Paced	536 (23.6)	42 (14.0)	83 (27.9)	113 (38.4)	<0.0001
Current NYHA functional class					<0.0001
II	1,477 (63.4)	218 (71.9)	204 (67.1)	147 (48.5)	
III	831 (35.6)	83 (27.4)	99 (32.6)	147 (48.5)	
IV	23 (1.0)	2 (0.7)	1 (0.3)	9 (3.0)	
KCCQ symptom stability score					0.8279
<50	186 (8.0)	28 (9.3)	20 (6.6)	19 (6.3)	
50	1,704 (73.5)	213 (70.8)	223 (73.6)	223 (74.3)	
>50	427 (18.4)	60 (19.9)	60 (19.8)	58 (19.3)	
Diabetes	748 (32.1)	57 (18.8)	104 (34.2)	138 (45.5)	<0.0001
Myocardial infarction	979 (42.0)	63 (20.8)	145 (47.7)	167 (55.1)	<0.0001
Hypertension	1,388 (59.9)	168 (55.8)	199 (65.7)	212 (70.2)	0.0008
Current smoker	388 (16.7)	47 (15.6)	53 (17.5)	48 (15.9)	0.7856
Body mass index, kg/m^2	29.9 (26.0–35.1)	32.1 (27.4–37.7)	29.4 (26.4–35.1)	29.8 (25.1–34.0)	<0.0001
Medication and devices					
ACE inhibitor or ARB	2,199 (94.3)	293 (96.7)	296 (97.4)	277 (91.4)	0.0009
Beta-blocker	2,203 (94.5)	295 (97.4)	287 (94.4)	280 (92.4)	0.0002
Beta-blocker dose, mg (N = 2,183)	38 (25–50)	50 (25–50)	37.3 (19.0–50.0)	25 (13–50)	0.023
Aldosterone receptor antagonist	1,051 (45.1)	147 (48.5)	138 (45.4)	121 (39.9)	0.0989
Digoxin	1,046 (44.9)	132 (43.6)	148 (48.7)	155 (51.2)	0.1620
Loop diuretic	1,816 (77.9)	216 (71.3)	237 (78.0)	265 (87.5)	<0.0001
Loop diuretic dose, mg (N = 1,783)	40 (40–80)	40 (30–80)	40 (40–80)	80 (40–120)	<0.0001
Insertable cardioverter-defibrillator	938 (40.2)	93 (30.7)	142 (46.7)	160 (52.8)	<0.0001
Biventricular pacemaker	419 (18.0)	29 (9.6)	66 (21.7)	85 (28.1)	<0.0001
Laboratory values					
hs-TnT, mg/l	14.8 (8.1–24.9)	8.0 (4.6–13.6)	14.5 (10.0–22.6)	24.9 (15.9–39.0)	<0.0001
GDF-15, pg/ml	1,596 (970–2,622)	839 (645–970)	1,596 (1,364–1,836)	3,320 (2,622–4,952)	<0.0001
NT-proBNP, pg/ml	815 (341–1,805)	404 (176–911)	813 (378–1,526)	2,159 (900–4,577)	<0.0001
Creatinine, mg/dl	1.20 (1.00–1.50)	1.00 (0.90–1.20)	1.20 (1.00–1.50)	1.50 (1.20–1.80)	<0.0001
eGFR (MDRD) creatinine clearance, ml/min	66.4 (50.6–81.0)	79.9 (68.7–94.6)	66.4 (49.6–78.1)	49.0 (38.9–63.3)	<0.0001
Blood urea nitrogen, mg/dl	20 (15–28)	16 (13–19)	21 (15–26)	28 (21–41)	<0.0001
Hemoglobin, g/dl	13.5 (12.3–14.6)	13.5 (12.5–14.7)	13.4 (12.3–14.8)	12.9 (11.7–14.1)	<0.0001

Values are median (quartile range [Q1–Q3]) or n (%).

ACE-i = angiotensin-converting enzyme inhibitors; ARB = angiotensin receptor blockers; BB = beta blocker; BMI = body mass index; BP = blood pressure; CPX = cardiopulmonary exercise; eGFR = estimated glomerular filtration rate; HF = heart failure; hs-TnT = high sensitivity troponin T; HR = hazard ratio; IVCD = intraventricular conduction delay; KCCQ = Kansas City Cardiomyopathy Questionnaire; LBBB = left bundle branch block; LVEF = left ventricular ejection fraction; MDRD = modification of diet in renal disease; MI = myocardial infarction; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association; peak Vo_2 = maximal oxygen consumption; RBBB = right bundle branch block.

higher, again, consistent with more severe HF in the patients with the highest GDF-15 values. In the overall HF-ACTION cohort, some differences in baseline characteristics were seen among patients who were included and excluded from multivariate modeling due to missing data; however, missing data did not have an impact on clinical outcomes (data not shown).

TABLE 2 Association of GDF-15 With Baseline Clinical and Biomarker Variables

	Coefficient	95% Confidence Limits	T Statistic	p Value
Log-2 (hs-TnT)	0.18	0.12 to 0.23	6.32	<0.0001
Age, yrs	0.01	0.01 to 0.02	5.86	<0.0001
Peak Vo_2, ml/kg/min	−0.04	−0.06 to −0.03	−5.69	<0.0001
Log-2 (creatinine)	0.35	0.22 to 0.48	5.25	<0.0001
Log-2 (NT-proBNP)	0.08	0.05 to 0.12	4.39	<0.0001
LVEF, %	0.01	0.007 to 0.02	3.75	0.0002
SBP, mm Hg for values up to 125	−0.008	−0.01 to −0.003	−3.49	0.0005
SBP, mm Hg for values above 125	0.008	0.002 to 0.01	2.47	0.0136
White race	0.19	0.08 to 0.31	3.29	0.0011
BMI, for values up to 35 kg/m^2	−0.02	−0.03 to −0.008	−3.20	0.0015
Blood urea nitrogen, mg/dl	0.007	0.002 to 0.01	2.98	0.0030
Diabetes	0.17	0.06 to 0.28	2.96	0.0032
Heart rate, beats/min	0.006	0.001 to 0.01	2.64	0.0086
NYHA functional class III/IV	0.12	0.01 to 0.23	2.19	0.0292

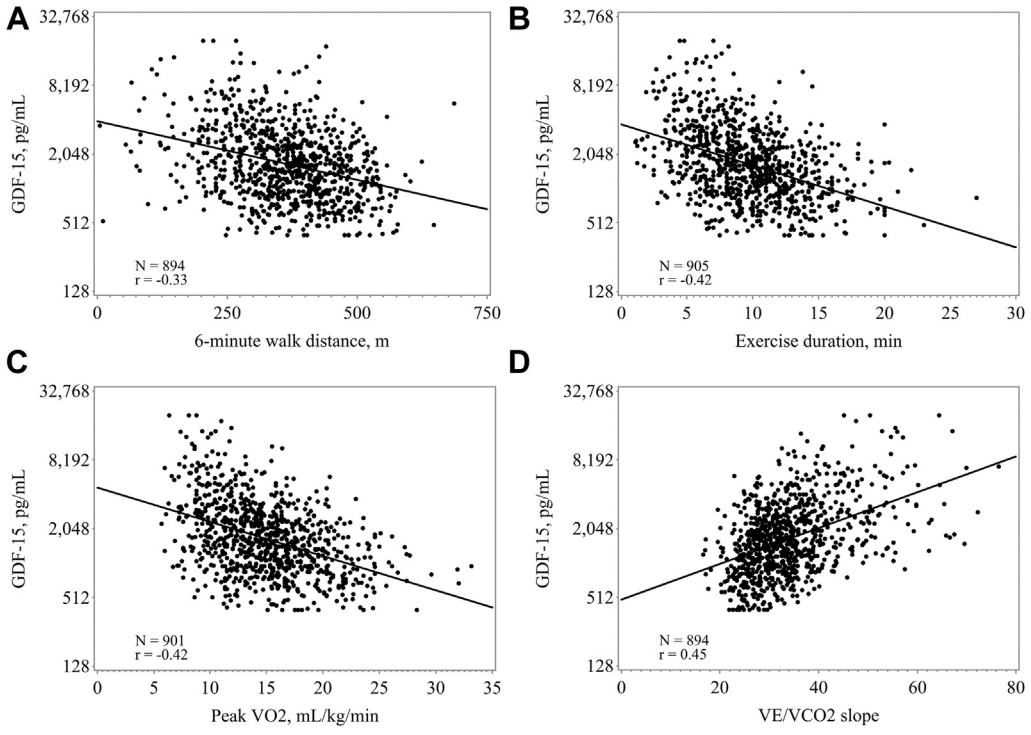
SBP = systolic blood pressure; Vo_2 = maximum rate of oxygen consumption; other abbreviations as in Table 1.

BIOLOGICAL CORRELATES OF GDF-15. The independent variable most strongly associated with higher GDF-15 concentration was higher hs-TnT ($p < 0.0001$) (Table 2). In addition, older age, lower peak Vo_2, and higher creatinine and NT-proBNP concentrations were also found to be associated with higher GDF-15 concentrations after multivariate adjustment.

GDF-15 AND SYMPTOM STATUS, FUNCTIONAL CAPACITY, AND EXERCISE PERFORMANCE. Higher GDF-15 concentrations were consistently associated with shorter 6-min walk distances, lower exercise durations, lower peak Vo_2, and higher V_E/Vo_2 slope (Figure 1). Those in the highest tertile of GDF-15 had the lowest exercise duration and lowest peak Vo_2 (Table 1). When we assessed higher GDF-15 concentration as a continuous variable, it was associated with a lower baseline peak Vo_2 ($r = −0.40$; $p < 0.0001$) and shorter 6-min walk distance ($r = −0.32$; $p < 0.0001$).

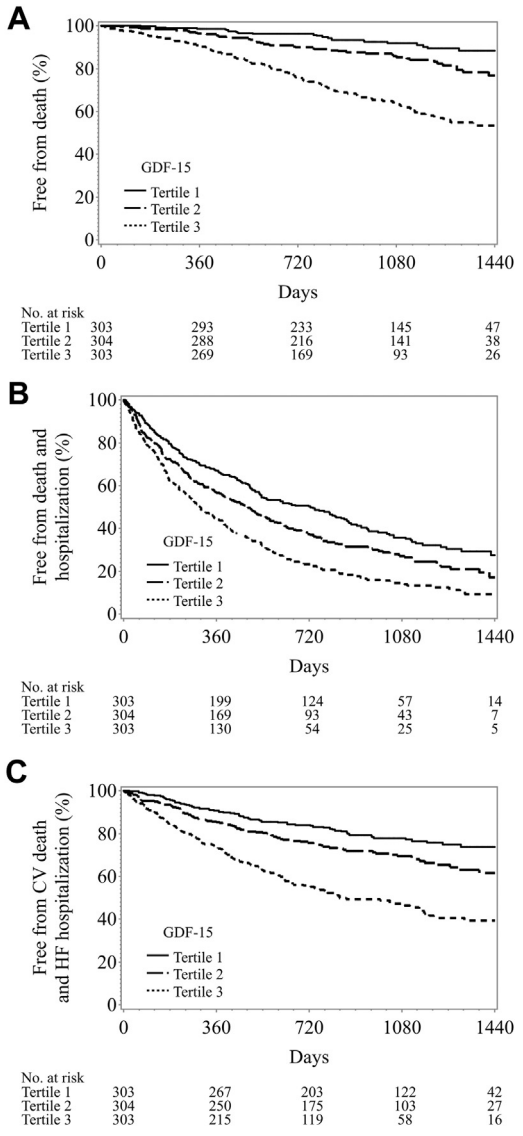
GDF-15 AND CLINICAL ENDPOINTS. The median follow-up was 32, months and there were 171 deaths, 647 all-cause deaths or hospitalization events, and

FIGURE 1 GDF-15 and Symptom Status, Functional Capacity, and Exercise Performance



Associations among growth differentiation factor (GDF)-15 and **(A)** 6-min walk distance, **(B)** exercise duration, **(C)** peak Vo_2, and **(D)** VE/Vco_2 slope. Vo_2 = maximal oxygen consumption; VE/Vco_2 slope = minute ventilation/carbon dioxide production.

FIGURE 2 Unadjusted Kaplan-Meier Curves of GDF-15 Tertiles



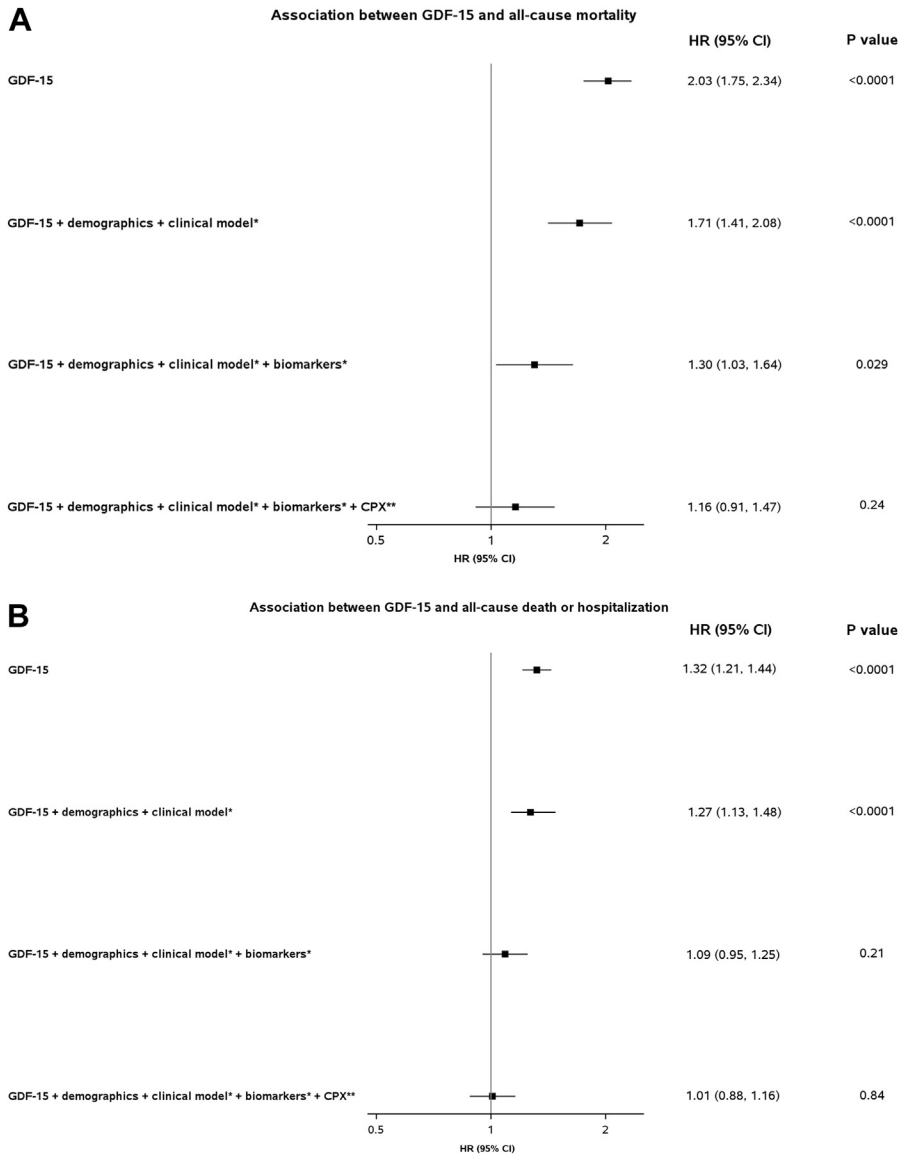
Growth differentiation factor (GDF)-15 levels at lowest tertile: <1,173 pg/ml; at middle tertile: 1,173 to 2,252 pg/ml; at highest tertile: >2,252 pg/ml. **(A)** All-cause death. **(B)** All-cause death and hospitalization. **(C)** Cardiovascular death and heart failure hospitalization.

301 cardiovascular deaths or HF hospitalization events in our study cohort. Generally, higher tertiles of GDF-15 were associated with worse clinical outcomes across all clinical endpoints of interest (Figure 2). In univariate analyses, a 2-fold increased concentration of GDF-15 was associated with a 203% increase in all-cause mortality, a 32% increase in the composite of all-cause death or all-cause hospitalization, and a 61% increase in the composite of cardiovascular death or HF hospitalization ($p < 0.0001$) for all.

In order to assess the independent association of GDF-15 in the context of other common clinically available data, we created a series of multivariate models that sequentially included clinical and demographic variables, other guideline-recommended biomarkers (NT-proBNP and hs-TnT), and variables obtained from CPX testing. After we adjusted for demographic and clinical variables only, we found a 2-fold increase in the concentration of GDF-15 was associated with a 71% increased risk of all-cause death ($p < 0.0001$) (Figure 3A), a 27% increased risk in all-cause death or rehospitalization ($p < 0.0001$) (Figure 3B), and a 34% increased risk of cardiovascular death or HF rehospitalization ($p = 0.0005$) (Figure 3C). After additionally adjusting for both hs-TnT and NT-proBNP in addition to clinical and demographic variables, we found a doubling of the baseline concentration of GDF-15 was still associated with a 30% increased risk of all-cause death ($p = 0.03$) (Figure 3A). After we made further adjustments for CPX variables in addition to the clinical model and biomarkers, GDF-15 was no longer significantly associated with any of the clinical endpoints of interest. There were no significant interactions between GDF-15 and randomized treatment assignment (exercise training vs. control) for any of the clinical endpoints (all: $p > 0.14$), suggesting that GDF-15 did not identify patients more or less likely to respond to exercise training.

PROGNOSTIC UTILITY OF GDF-15. The prognostic utility of GDF-15 in addition to the clinical models, biomarkers (NT-proBNP and hs-TnT), and CPX variables are shown in Table 3. GDF-15 improved discrimination of all-cause death in addition to the clinical model, particularly the discrimination of nonevents. When we compared the addition of biomarkers to the clinical model, all biomarker improved discrimination of all-cause death individually. However, NT-proBNP appears to have the greatest magnitude of improvement in discrimination, followed by GDF-15 (Online Table 2). Although the addition of GDF-15 incrementally increased the c-index value for all-cause death in addition to clinical, biomarker, and CPX variables, the improvement was not significant overall. Similarly, GDF-15 did not significantly improve discrimination for the composite outcomes of all-cause death or hospitalization and cardiovascular death or HF hospitalization.

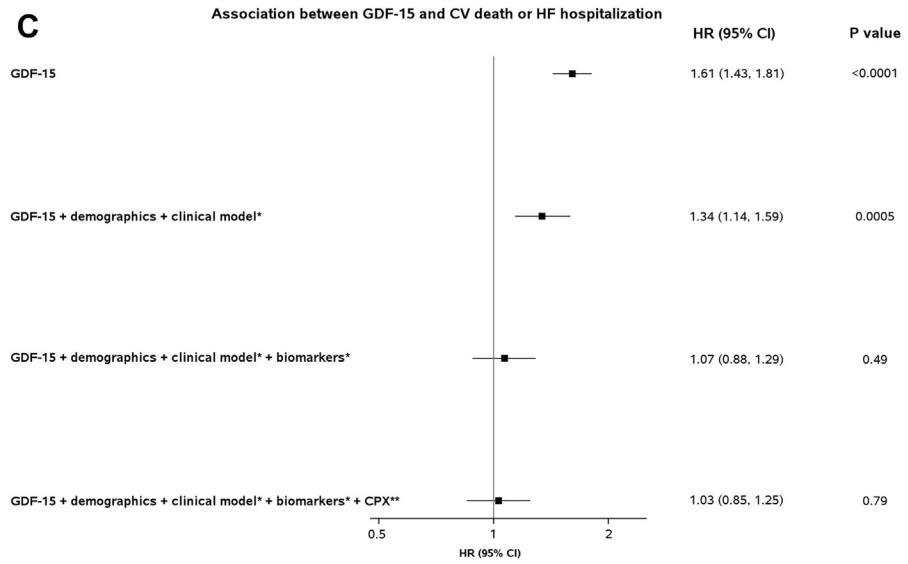
FIGURE 3 Full Adjusted Model and Adjusted Clinical Models



(A) Full model adjusted for demographic variables and serum creatinine concentration (truncated at 2.3 mg/dL), BMI, loop diuretic dose (truncated at 100 mg furosemide equivalents), CCS angina classes (0, I, ≥II), and LVEF. *Biomarkers refer to NT-proBNP and hs-TnT; **CPX refers to baseline cardiopulmonary exercise stress test variables, which include exercise duration and ventricular conduction. **(B)** Clinical model adjusted for KCCQ symptom stability score (3 categories: <50, 50, >50), U.S. BUN concentration, LVEF, BB dose (truncated at 50 mg metoprolol equivalents), moderate or severe MR. *Biomarkers refer to NT-proBNP and hs-TnT; **CPX refers to baseline cardiopulmonary exercise stress test variables, which include peak V_{O_2} characterized by Weber class and ventricular conduction on the baseline CPX test. **(C)** Clinical model adjusted for loop diuretic dose (truncated at 100), LVEF, moderate or severe MR, KCCQ symptom stability score (3 categories: <50, 50, >50), BUN concentration (truncated at 39 mg/dL). *Biomarkers refer to NT-proBNP and hs-TnT; **CPX refers to baseline cardiopulmonary exercise stress test variables, which include peak V_{O_2} characterized by Weber class, VE/V_{CO_2}, and ventricular conduction on the baseline CPX test. BB = beta blocker; BMI = body mass index; BUN = blood urea nitrogen; CCS = Canadian Cardiovascular Society classification; CPX = cardiopulmonary exercise test; hs-TnT = high sensitivity troponin T; KCCQ = Kansas City Cardiomyopathy Questionnaire; LVEF = left ventricular ejection fraction; MR = mitral regurgitation; NT-proBNP = N-terminal pro-B-type natriuretic peptide; other abbreviations as in Figure 1.

Continued on the next page

FIGURE 3 Continued



DISCUSSION

Assessing the clinical utility of new and existing biomarkers remains an important goal in HF. The present analyses from the HF-ACTION study adds a number of important findings regarding the role between GDF-15 and HF: 1) a 2-fold greater concentration of GDF-15 is associated with a 30% greater risk of all-cause death, despite comprehensive multivariate adjustment including hs-TnT and NT-proBNP; 2) greater GDF-15 concentrations were associated with worse symptom burden and functional capacity, including baseline NYHA functional class, 6-min walk distance, and peak Vo_2; and 3) the strongest baseline predictor of higher GDF-15 concentrations was greater hs-TnT. Because it was a large multicenter cohort of demographically diverse, ambulatory, chronic HFrEF patients, the HF-ACTION trial allowed for a comprehensive assessment of GDF-15's association with cardiovascular outcomes. The range of outcomes in HF-ACTION, including CPX data available through HF-ACTION, provides a unique opportunity to evaluate the association between GDF-15, functional capacity, and exercise performance.

ASSOCIATION OF GDF-15 WITH BASELINE CLINICAL AND BIOMARKER VARIABLES. Cellular expression of GDF-15 increases in response to various stressors including reactive oxygen species and proinflammatory cytokines (8,21). In murine models, GDF-15 inhibits apoptosis, hypertrophy, and adverse cardiac remodeling (8,9,22). GDF-15 is not normally expressed in the heart; however, it can be induced in experimental models of myocardial ischemia, pressure/volume overload, and dilated cardiomyopathy (8,9). Our study expands upon these findings: we identified the fact that GDF-15 concentrations were closely associated with hs-TnT and NT-proBNP (**Table 2**), suggesting that cardiac ischemia and cardiomyocyte stretch play a role in GDF-15 expression. Our study also aligns with previous studies suggesting that GDF-15 is closely associated with multiple biologic pathways including renal dysfunction and abnormal glucose regulation (11,16,23,24).

ASSOCIATION BETWEEN GDF-15 AND CARDIOVASCULAR OUTCOMES. Current HF guidelines indicate that natriuretic peptides and biomarkers of myocardial injury (cardiac troponin T or I) may be considered for ambulatory risk stratification (5). Our findings suggest that GDF-15 may also play an important role in ambulatory risk stratification. In the ValHeFT analysis, after adjustment for clinical and biomarker variables, an increase of 100 pg/ml GDF-15 was associated with an increased risk of all-cause death (HR: 1.007; 95% CI: 1.001 to 1.014; $p = 0.02$) (16). Compared to that of ValHeFT, our patient population was younger (median 59 vs. 63 years of age, respectively), had increased nonwhite participants (36% vs. 16%, respectively), a higher proportion of patients

TABLE 3 Prognostic Utility of GDF-15

Model	C Statistic Without GDF-15	C Statistic With GDF-15	Overall NRI
All-cause death			
Clinical model	**0.704 (0.659 to 0.749)**	**0.735 (0.690 to 0.779)**	**0.298 (0.081 to 0.516)**
Clinical model + CPX	0.751 (0.709 to 0.794)	0.759 (0.717 to 0.801)	0.150 (−0.071 to 0.377)
Clinical model + biomarkers	0.755 (0.713 to 0.797)	0.759 (0.717 to 0.801)	0.105 (−0.124 to 0.339)
Clinical model + biomarkers + CPX	0.770 (0.730 to 0.810)	0.772 (0.732 to 0.812)	−0.038 (−0.263 to 0.192)
All-cause death or hospitalization			
Clinical model	0.612 (0.584 to 0.640)	0.623 (0.595 to 0.650)	0.090 (−0.242 to 0.378)
Clinical model + CPX	0.637 (0.610 to 0.664)	0.640 (0.613 to 0.668)	−0.181 (−0.500 to 0.111)
Clinical model + biomarkers	0.636 (0.609 to 0.663)	0.639 (0.612 to 0.666)	−0.272 (−0.632 to 0.039)
Clinical model + biomarkers + CPX	0.649 (0.623 to 0.676)	0.650 (0.623 to 0.676)	−0.404 (−0.737 to −0.102)
CV death or HF hospitalization			
Clinical model	0.715 (0.680 to 0.749)	0.725 (0.691 to 0.759)	0.111 (−0.086 to 0.317)
Clinical model + CPX	0.730 (0.696 to 0.764)	0.733 (0.700 to 0.767)	0.028 (−0.168 to 0.238)
Clinical model + biomarkers	0.752 (0.720 to 0.784)	0.752 (0.719 to -0.784)	−0.063 (−0.250 to 0.144)
Clinical model + biomarkers + CPX	0.755 (0.723 to 0.787)	0.756 (0.723 to 0.788)	−0.084 (−0.281 to 0.123)

TABLE 3 Continued

Model	NRI for Events	NRI for Nonevents	IDI
All-cause death			
Clinical model	**0.044 (−0.131 to 0.227)**	**0.254 (0.155 to 0.342)**	**0.043 (0.025 to 0.062)**
Clinical model + CPX	−0.029 (−0.201 to 0.150)	0.179 (0.087 to 0.272)	0.009 (−0.002 to 0.019)
Clinical model + biomarkers	0.058 (−0.116 to 0.236)	0.046 (−0.050 to 0.140)	0.004 (−0.005 to 0.011)
Clinical model + biomarkers + CPX	−0.044 (−0.217 to 0.136)	0.006 (−0.099 to 0.106)	−0.001 (−0.005 to 0.004)
All-cause death or hospitalization			
Clinical model	−0.030 (−0.122 to 0.066)	0.120 (−0.164 to 0.369)	0.008 (0.002 to 0.015)
Clinical model + CPX	−0.101 (−0.190 to −0.002)	−0.080 (−0.379 to 0.170)	0.002 (−0.002 to 0.006)
Clinical model + biomarkers	−0.105 (−0.193 to −0.009)	−0.168 (−0.490 to 0.099)	−0.004 (−0.007 to −0.002)
Clinical model + biomarkers + CPX	−0.144 (−0.234 to −0.046)	−0.260 (−0.569 to −0.007)	0.000 (−0.001 to 0.000)
CV death or HF hospitalization			
Clinical model	0.013 (−0.113 to 0.155)	0.098 (−0.015 to 0.219)	0.007 (0.000 to 0.015)
Clinical model + CPX	−0.028 (−0.159 to 0.118)	0.056 (−0.061 to 0.177)	0.000 (−0.005 to 0.004)
Clinical model + biomarkers	−0.070 (−0.201 to 0.079)	0.007 (−0.106 to 0.123)	−0.001 (−0.003 to 0.000)
Clinical model + biomarkers + CPX	−0.091 (−0.230 to 0.057)	0.007 (−0.106 to 0.123)	−0.001 (−0.001 to 0.000)

Bold text indicates results where GDF-15 significantly increased the C-statistic, overall NRI, and IDI.
CV = cardiovascular; HF = heart failure; IDI = integrated discriminatory improvement; NRI = net reclassification index; other abbreviations as in Table 1.

with hypertension (64% vs. 7%, respectively), lower median GDF-15 concentrations (1,596 pg/ml vs. 2,040 pg/ml, respectively), and greater use of evidence-based medical therapy (use of beta-blocker therapy: 95% vs. 33%, respectively; and use of spironolactone: 45% vs. 2%, respectively). Despite these differences, our study still demonstrated that, in a demographically diverse, well-medically managed group of chronic HF patients, GDF-15 was associated with an increased risk of all-cause mortality, even after adjusting for commonly available demographic, clinical, and laboratory variables.

Compared to other biomarkers, GDF-15 appears to have a robust association with all-cause mortality. Prior studies from HF-ACTION have evaluated the fibrosis marker galectin-3 (25) and the interleukin signaling ligand-soluble ST-2 (26). Galectin-3 was not associated with an increased risk of all-cause death after adjustment for clinical variables and NT-proBNP (adjusted HR: 1.06; p = 0.30) (25). A doubling of ST-2 was associated with increased risk of all-cause death (adjusted HR: 1.42; p = 0.007) (26); however, this study did not adjust for troponin, which is a powerful predictor of mortality in patients with HF (26).

In our study, GDF-15 did not provide any additional prognostic information when CPX variables were added to demographic, clinical, laboratory, and biomarker data. Similar results have been seen with other biomarkers, including ST-2 and galectin-3 (25,26). Our results suggest that the unique prognostic information provided by GDF-15 is also captured by variables derived from CPX testing.

Abnormal CPX measurements indicate worse HF and may reduce the association between GDF-15 and cardiovascular outcomes. As CPX testing is not widely available or performed in the routine management of HF patients in clinical practice (27), our results demonstrate the strength of GDF-15's association with clinical outcomes of interest in addition to routinely available clinical and biomarker variables.

GDF-15 significantly improved discrimination for all-cause mortality in addition to clinical variables; however, when CPX variables or additional biomarkers were added to the clinical model for all-cause death, model discrimination did not improve after the addition of GDF-15. In the ValHeFT study, the addition of GDF-15 to clinical and biomarker variables (BNP, high sensitivity c-reactive protein, and hs-TnT) did improve the c-index for mortality (c-index value from 0.73 to 0.76; $p = 0.02$), and the IDI for mortality was borderline significant ($p = 0.06$) (16). Among patients with chronic HF in a Singapore cohort, GDF-15 also improved discrimination in addition to a clinical model with NT-proBNP and hs-TnT (c-index from 0.72 to 0.74; $p = 0.0019$) (28). In our study, the discriminatory ability of biomarkers (hs-TnT and NT-proBNP) and CPX variables in addition to baseline clinical variables for the outcome of all-cause mortality was already high, as reflected by a c-index of 0.77 (**Table 3**). Although GDF-15 improved the c-index for all-cause mortality, in addition to baseline biomarkers and CPX variables, this increase was not significant. This finding likely reflects the challenge of improving discrimination in addition to an already robust model. However, combined with the prior analyses of GDF-15 among patients with HF, the totality of evidence suggest that GDF-15 may improve discrimination above and beyond clinical variables and established biomarkers.

STUDY LIMITATIONS. This was a post hoc study and was subject to the limitations of these types of analyses. Patients enrolled in the biomarker substudy were predominantly from the United States. As with most clinical trials, our patient population differed from the broader HF population seen in HF registries (29), although HF-ACTION was notable for enrolling a higher proportion of nonwhite patients and women than most other HF clinical trials. Furthermore, the patients in HF-ACTION demonstrated higher use of evidence-based therapies than those in other studies evaluating the prognostic role of GDF-15 among patients with HF. Attempting to assess the association of GDF-15 and individual outcomes relative to an optimized set of variables might have contributed to the difficulties in showing a significant association with outcomes besides all-cause mortality. Patients enrolled in the HF-ACTION trial had impaired ejection fraction (LVEF: <0.35%); as such our results cannot be extrapolated to other HF populations such as patients with HF and preserved ejection fraction. Our study revealed that patients with higher GDF-15 had worse peak Vo_2; however, all patients had to consent and be able to perform a CPX test, which may not reflect the overall HF population.

CONCLUSIONS

In this analysis of a large clinical study with well-treated chronic HFrEF patients, increasing baseline GDF-15 was associated with long-term adverse cardiovascular outcomes. Even after comprehensive multivariate adjustment, including demographics, clinical variables, and biomarkers (hs-TnT and NT-proBNP), doubling the concentration of GDF-15 was significantly associated with all-cause mortality. Further adjustment for CPX-derived variables attenuated the value of GDF-15, but CPX testing is not widely used in routine clinical practice. These data suggest a potential role for GDF-15 in risk stratification among patients with chronic HFrEF.

ADDRESS FOR CORRESPONDENCE: Dr. G. Michael Felker, Duke Clinical Research Institute, 2400 Pratt Street, Durham, North Carolina 27705. E-mail: Michael.felker@dm.duke.edu.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: GDF-15, a member of the transforming growth factor-β cytokine family, is an emerging biomarker used for risk stratification among patients with cardiovascular diseases. In our analysis of a demographically diverse, well-managed patients with chronic HFrEF, GDF-15 provides independent prognostic information in addition to established predictors of outcomes including hs-TnT and NT-proBNP.

TRANSLATIONAL OUTLOOK: Our findings suggest a possible role for GDF-15 in the risk stratification of patients with chronic HFrEF. Future studies evaluating the mechanistic role of GDF-15 in patients with HF may lead to new therapeutic targets.

REFERENCES

1. Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002;347:161–7.

2. Writing Group Members, Mozaffarian D, Benjamin EJ, et al. Heart disease and stroke statistics-2016 update: a report from the American Heart Association. Circulation 2016;133:e38–60.

3. Curtis LH, Whellan DJ, Hammill BG, et al. Incidence and prevalence of heart failure in elderly persons, 1994-2003. Arch Intern Med 2008;168:418–24.

4. Braunwald E. The war against heart failure: the Lancet lecture. Lancet 2015;385:812–24.

5. Yancy CW, Jessup M, Bozkurt B, et al. 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:e147–239.

6. Thygesen K, Mair J, Mueller C, et al. Recommendations for the use of natriuretic peptides in acute cardiac care: a position statement from the Study Group on Biomarkers in Cardiology of the ESC Working Group on Acute Cardiac Care. Eur Heart J 2012;33:2001–6.

7. Kempf T, Zarbock A, Widera C, et al. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. Nat Med 2011;17:581–8.

8. Kempf T, Eden M, Strelau J, et al. The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. Circ Res 2006;98:351–60.

9. Xu J, Kimball TR, Lorenz JN, et al. GDF-15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. Circ Res 2006;98:342–50.

10. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2016;37:2129–200.

11. Wallentin L, Hijazi Z, Andersson U, et al. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial. Circulation 2014;130:1847–58.

12. Wollert KC, Kempf T, Lagerqvist B, Lindahl B. Growth differentiation factor 15 for risk stratification and selection of an invasive treatment strategy in non-st-elevation acute coronary syndrome. Circulation 2007;116:1540–8.

13. Daniels LB, Clopton P, Laughlin GA, Maisel AS, Barrett-Connor E. Growth-differentiation factor-15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: the Rancho Bernardo study. Circulation 2011;123:2101–10.

14. Eggers KM, Kempf T, Lagerqvist B, et al. Growth-differentiation factor-15 for long-term risk prediction in patients stabilized after an episode of nonST-segment-elevation acute coronary syndrome. Circ Cardiovasc Genet 2010;3:88–96.

15. Dallmeier D, Brenner H, Mons U, Rottbauer W, Koenig W, Rothenbacher D. Growth differentiation factor 15, its 12-month relative change, and risk of cardiovascular events and total mortality in patients with stable coronary heart disease: 10-year follow-up of the KAROLA Study. Clin Chem 2016;62:982–92.

16. Anand IS, Kempf T, Rector TS, et al. Serial measurement of growth-differentiation factor-15 in heart failure: relation to disease severity and prognosis in the valsartan heart failure trial. Circulation 2010;122:1387–95.

17. Whellan DJ, O'Connor CM, Lee KL, et al. Heart Failure and A Controlled Trial Investigating Outcomes of Exercise TraiNing (HF-ACTION): design and rationale. Am Heart J 2007;153:201–11.

18. O'Connor CM, Whellan DJ, Lee KL, et al. Efficacy and safety of exercise training in patients with chronic heart failure: HF-ACTION randomized controlled trial. JAMA 2009;301:1439–50.

19. Wollert KC, Kempf T, Giannitsis E, et al. An automated assay for growth differentiation factor 15. J App Lab Med 2017;1:510–21.

20. O'Connor CM, Whellan DJ, Wojdyla D, et al. Factors related to morbidity and mortality in patients with chronic heart failure with systolic dysfunction: the HF-ACTION predictive risk score model. Circ Heart Fail 2012;5:63–71.

21. Januzzi JL, Camargo CA, Anwaruddin S, et al. The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. Am J Cardiol 2005;95:948–54.

22. Kim H-N, Januzzi JL. Natriuretic peptide testing in heart failure. Circulation 2011;123:2015–9.

23. Kempf T, Horn-Wichmann R, Brabant G, et al. Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. Clin Chem 2006;53:284–91.

24. Kempf T, Haehling von S, Peter T, et al. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. J Am Coll Cardiol 2007;50:1054–60.

25. Felker GM, Fiuzat M, Shaw LK, et al. Galectin-3 in ambulatory patients with heart failure: results from the HF-ACTION study. Circ Heart Fail 2012;5:72–8.

26. Felker GM, Fiuzat M, Thompson V, et al. Soluble ST-2 in ambulatory patients with heart failure: association with functional capacity and long-term outcomes. Circ Heart Fail 2013;6:1172–9.

27. Balady GJ, Arena R, Sietsema K, et al. Clinician's guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. Circulation 2010;122:191–225.

28. Chan MMY, Santhanakrishnan R, Chong JPC, et al. Growth differentiation factor 15 in heart failure with preserved vs. reduced ejection fraction. Eur J Heart Fail 2015;18:81–8.

29. Fonarow GC, Albert NM, Curtis AB, et al. Improving evidence-based care for heart failure in outpatient cardiology practices: primary results of the registry to Improve the Use of Evidence-Based Heart Failure Therapies in the Outpatient Setting (IMPROVE HF). Circulation 2010;122:585–96.

KEY WORDS BNP, B-type natriuretic peptide, heart failure, N-terminal pro-B-type natriuretic peptide

APPENDIX For supplemental tables, please see the online version of this article.