Published in final edited form as:

Am Heart J. 2020 February ; 220: 97–107. doi:10.1016/j.ahj.2019.11.006.

Are Existing and Emerging Biomarkers Associated with Cardiorespiratory Fitness in Patients with Chronic Heart Failure?

Marat Fudim, MD, MHS^{1,2}, Jacob P. Kelly, MD, MHS³, Aaron D. Jones, M.Biostat.¹, Omar AbouEzzeddine, MD⁴, Andrew P. Ambrosy, MD⁵, Stephen J. Greene, MD^{1,2}, Yogesh N. V. Reddy, MBBS⁴, Kevin J. Anstrom, PhD¹, Brooke Alhanti, PhD¹, Gregory D. Lewis, MD⁶, Adrian F. Hernandez, MD, MHS^{1,2}, G. Michael Felker, MD^{1,2}

¹Duke Clinical Research Institute and Division of Cardiology, Durham, NC

²Division of Cardiology, Duke University Medical Center, Durham, NC

³Heart and Vascular Institute, Anchorage, AL

⁴Department of Cardiovascular Diseases, Mayo Clinic, Rochester, MN

⁵Division of Research, Kaiser Permanente Northern California, Oakland, CA

⁶Division of Cardiology, Massachusetts General Hospital, Boston, MA

Abstract

Aims: Cardiorespiratory fitness (CRF) is closely linked to health status and clinical outcomes in heart failure (HF) patients. We aimed to test whether biomarkers can reflect CRF and its change over time.

Methods and Results: This *post-hoc* analysis utilized data from ambulatory cohorts of heart failure with reduced ejection fraction (HFrEF) (IRONOUT) and heart failure with preserved ejection fraction (HFpEF) (RELAX). Cardiopulmonary exercise testing, 6 minute walk distance (6MWD), and serum biomarkers were measured at baseline and 16 or 24 week follow-up (for IRONOUT and RELAX respectively). Biomarkers included N-terminal pro-B-type natriuretic peptide (NT-proBNP), soluble ST2, growth differentiation factor-15 (GDF-15), and galectin-3. Analysis included 225 patients with HFrEF and 216 with HFpEF. Baseline peak VO_2 , VE/VCO_2 slope and 6MWD showed a mild correlation with the doubling of all four tested biomarkers in HFrEF and HFpEF. Following multivariable adjustment (including all biomarkers), the only significant association between change in biomarker and functional parameter in HFrEF was change in NT-proBNP and change in VE/VCO_2 slope (3.596% increase per doubling, 95% CI [0.779, 6.492]; $p=0.012$). In HFpEF a decrease in peak VO_2 was associated with an increase in NT-proBNP (-0.726 ml/min/kg per doubling, 95% CI [-1.100 , -0.353]; $p<0.001$) and a decrease

Corresponding Author: Marat Fudim, MD, 2301 Erwin Road, 27710 Durham, NC, Tel: (919) 668-8917; Fax: (919) 668-3575; marat.fudim@duke.edu.

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in 6MWD was associated with an increase in GDF-15 (-31.606 m per doubling, 95% CI $[-61.404, -1.809]$; $p=0.038$).

Conclusions: In these ambulatory trial cohorts, NT-proBNP was associated with baseline and change in CRF in HFrEF and HFpEF. In contrast, novel biomarkers do not appear suitable as a reliable surrogate for serial assessment of exercise capacity in HF patients given lack of consistent independent association with CRF beyond traditional risk factors and NT-proBNP.

Clinical Trial Registration: and

Keywords

Heart failure; preserved ejection fraction; reduced ejection fraction; biomarkers; exercise function; cardiorespiratory fitness

INTRODUCTION

Among patients with heart failure (HF), cardiorespiratory fitness as measured by cardiopulmonary exercise testing (CPET) or the 6 minute walk test are powerful predictors of morbidity and mortality [1-4]. Cardiorespiratory fitness is a key objective measure recommended for risk stratification during evaluation for advanced HF therapies [5]. Further, cardiorespiratory fitness is a variable that is responsive to various therapies in patients with chronic HF (exercise training, medications, mechanical circulatory devices) [5-8], thus supporting the value of serial measurements of cardiorespiratory fitness. A decrease in cardiorespiratory fitness between examinations is strongly associated with a higher risk of adverse patient-reported and clinical outcomes, when compared to patients experiencing an increase in cardiorespiratory fitness [9]. However, performing standardized functional testing in patients as part of routine clinical care, especially CPET, is complicated by multiple barriers such as need for equipment, trained personnel, time, and patient effort.

A series of clinically established and novel biomarkers of cardiovascular stress, inflammation, and fibrosis in patients with HF have been associated with adverse clinical outcomes and are either used or proposed for use as risk predictors [10]. Serial testing of biomarkers to help guide HF management, whether for medical/decongestive therapies or repeat risk stratification, has been investigated retrospectively and prospectively with mixed results [11-14]. Nonetheless, whether biomarkers can serve as reliable and practical alternative to serial CPET for longitudinal monitoring of patient status remains to be determined [15, 16]. Moreover, data linking traditional cardiorespiratory fitness measures to specific biomarkers may invoke biologic processes most responsible for impaired functional status, such as filling pressures and wall stress (N-terminal pro-B-type natriuretic peptide [NT-proBNP]), hypertrophy and fibrosis (soluble ST2, [ST2] and galectin 3), and inflammation (growth differentiation factor [GDF-15]). Identifying a potential link between cardiopulmonary performance and novel biomarkers may also inform our understanding of potential (differential) pathophysiological relationships between key HF subgroups such as heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF).

In the present analysis, we sought to test the association between biomarkers and cardiorespiratory fitness at baseline and during follow up in two well characterized chronic HF clinical trial cohorts from the Heart Failure Network (HFN) trials: the *IRONOUT* (Iron Repletion effects ON Oxygen UpTake in Heart Failure) trial of patients with HFrEF, and the *RELAX* (Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Heart Failure with Preserved Ejection Fraction) trial of patients with HFpEF.

METHODS

Statistical Analysis

Overview—This *post-hoc* analysis was performed using data from the National Heart, Lung, and Blood Institute (NHLBI)-sponsored HFN *IRONOUT* and *RELAX* trials (Figure 1). Each protocol was approved by the Institutional Review Boards at each site, and written informed consent was obtained from all patients before randomization. *IRONOUT* was a double-blind, placebo-controlled, randomized trial designed to test the efficacy and safety of oral iron polysaccharide compared to placebo among patients with HFrEF (defined as an EF <40% and New York Heart Association [NYHA] functional class II-IV symptoms) and iron deficiency (i.e. defined as a ferritin 15-100 ng/mL or ferritin 100-299 ng/mL with a transferrin saturation <20%)[17]. *RELAX* was a double-blind, randomized trial designed to test the effect of the phosphodiesterase-5 inhibitor sildenafil compared with placebo on exercise capacity and clinical status in HFpEF. *RELAX* enrolled ambulatory patients who had an EF ≥50% and objective evidence of HF [18, 19]. Subjects were required to have elevated N-terminal pro-B-type natriuretic peptide (NT-proBNP, ≥400 pg/mL) or elevated invasively measured filling pressures and reduced exercise capacity (<60% age-, sex- and body size-specific predicted VO₂). In both trials, patients with advanced chronic kidney disease were excluded (estimated glomerular filtration rate <20 mL/min/1.73m²).

Biomarkers and Exercise Testing—Participants underwent baseline studies including a history and physical examination, echocardiography, CPET, six-minute walk test, and serum biomarkers. CPET and biomarker measurements were performed at baseline and follow-up at 16 weeks in *IRONOUT* and 24 weeks in *RELAX*. In both studies, CPET was performed using an identical protocol and interpreted by the HFN CPET core laboratory (Massachusetts General Hospital, Boston, MA) as previously reported [19, 20]. Patients unable to achieve a respiratory exchange ratio greater than or equal to 1.0 on baseline screening CPET were excluded. Plasma biomarker measurements were performed by the HFN biomarker core laboratory (University of Vermont, Burlington, VT) as previously described [19, 20] and included NT-proBNP (i.e., marker of filling pressures and wall stress), ST2 (i.e., cardiovascular stress and fibrosis), GDF-15 (i.e., inflammation) and Galectin 3 (i.e., mediator of inflammation and fibrosis).

Statistics—Baseline characteristics were summarized with medians and quartiles for continuous variables and counts and percentages for categorical variables. Multiple imputation by chained equations was used to impute missing biomarkers and covariates for the regression models, which were aggregated across 100 imputed datasets. Missing outcomes were not imputed; patients without a baseline or follow-up outcome were omitted

from the corresponding model. Imputation and regression were stratified by clinical trial/HF subgroup, with separate models for *IRONOUT* (HFrEF) and *RELAX* (HFpEF) patients.

This analysis investigated the associations between each of three exercise capacity outcomes (peak VO_2 , VE/VCO_2 slope, and 6-minute walk distance [6MWD]) and each of the four plasma biomarkers mentioned previously (NT-proBNP, ST2, GDF-15, and Galectin 3). The baseline values of the outcomes and biomarkers were compared cross-sectionally, and the changes in the outcomes from baseline to 16 (*IRONOUT*, HFrEF) or 24 (*RELAX*, HFpEF) weeks were compared to the corresponding changes in the biomarkers. Each outcome-biomarker association was evaluated in a univariable model, an adjusted model controlling for six pre-specified potential confounders (age, sex, body mass index, prior HF hospitalization in past year, systolic blood pressure, and blood urea nitrogen), and a multivariable model further controlling for the other biomarkers. All models also included a random intercept for study site. Variables were transformed as appropriate to address linearity and influential observations. All biomarkers were $\log_2$ transformed. Generalized linear regression models were fit using the gamma, inverse Gaussian, and normal distributions, and the distribution with the smallest Akaike information criterion was chosen; the normal distribution was selected in each case. Sensitivity analyses controlling for treatment and treatment-biomarker interactions were also conducted for the change-from-baseline models to assess the impact of omitting treatment, which had null effects in both original trials, from the primary models.

Two-sided p values <0.05 were considered statistically significant. Statistical analyses were completed using SAS software, version 9.4 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Baseline characteristics

This analysis included all 225 patients with HFrEF (*IRONOUT*) and 216 with HFpEF (*RELAX*). Patients in both trial cohorts were predominantly white and male with a mean age >60 years (Table 1). The HFpEF ambulatory cohort had more evidence of congestion and a greater comorbidity burden than the HFrEF cohort. As expected, the HFrEF cohort had a high rate of guideline-directed medication use but diuretic use was $>75\%$ in both cohorts. Both patient groups had markedly impaired baseline exercise capacity as measured by peak VO_2 , VE/VCO_2 slope, and 6MWD (Table 2). In both study groups, median levels of NT-proBNP and GDF-15 were above the upper limit of normal, whereas median levels for Galectin-3 and ST2 were within the reference range (Table 2/Supplement Table 1).

Relationship between functional capacity and biomarkers at baseline

Baseline peak VO_2 showed a weak correlation with the doubling of all four tested biomarkers across both trials (Figure 2). Similar, but less prominent, was a correlation between VE/VCO_2 slope and biomarkers at baseline (Supplement Figure 1). Finally, 6MWD was correlated with a doubling of biomarkers at baseline (Supplement Figure 2). In unadjusted and adjusted analysis, most biomarkers were significantly associated with functional capacity, whether measured by CPET or 6MWD (Supplement Table 2). Following

multivariable adjustment (accounting for all four biomarkers), in HFrEF, NT-proBNP and GDF-15 at baseline were significantly associated with baseline peak VO_2 (NT-proBNP: -0.344 mL/min/kg per doubling, 95% CI $[-0.585, -0.103]$, $p=0.005$; and GDF-15: -1.528 mL/min/kg per doubling, 95% CI $[-2.179, -0.876]$, $p<0.001$) and VE/ VCO_2 slope (NT-proBNP: 3.398% increase per doubling, 95% CI $[1.501, 5.330]$, $p<0.001$; and GDF-15: 5.370% increase per doubling, 95% CI $[0.244, 10.759]$, $p=0.040$) (Table 3, Figure 3). Only GDF-15 was associated with a lower 6MWD (-33.579 m per doubling, 95% CI $[-57.645, -9.513]$, $p=0.006$).

In HFpEF, NT-proBNP and GDF-15 were associated with peak VO_2 (NT-proBNP: -0.464 mL/min/kg per doubling, 95% CI $[-0.676, -0.251]$, $p<0.001$; and GDF-15: -1.010 mL/min/kg per doubling, 95% CI $[-1.487, -0.532]$, $p<0.001$). For VE/ VCO_2 slope only NT-proBNP was found to have a significant association. For 6MWD there was only an association with ST2 in the multivariable analysis (Table 3, Figure 3).

Relationship between change in functional capacity and change in biomarkers

Associations between changes in exercise functional parameters and parallel changes in biomarkers from baseline to follow up were evaluated in 196 patients with HFrEF (*IRONOUT*) and 189 with HFpEF (*RELAX*) with measured functional capacity at follow-up (Supplement Table 3). Univariable (unadjusted and adjusted) results are presented in Figure 3 and Supplement Table 4. Following a multivariable analysis including all biomarkers (Table 4, Figure 4), in patients with HFrEF, the only significant association between a biomarker and functional parameter was a change in NT-proBNP and a change in VE/ VCO_2 slope (3.596% increase per doubling, 95% CI $[0.779, 6.492]$; $p=0.012$). In HFpEF, an increase in NT-proBNP was associated with a decrease in peak VO_2 (-0.726 mL/min/kg per doubling, 95% CI $[-1.100, -0.353]$; $p<0.001$) and an increase in GDF-15 was associated with decrease in 6MWD (-31.606 m per doubling, 95% CI $[-61.404, -1.809]$; $p=0.038$).

The decision to omit treatment from the follow-up analyses was assessed in two ways. A sensitivity analysis in only placebo control patients was performed to check for consistency with the primary analyses in treated and placebo patients (Supplement Table 5, Figure 4). Further analyses adjusting for treatment and treatment-biomarker interactions found a statistically significant interaction only for Peak VO_2 vs. Galectin-3 in HFpEF (not shown). One significant result ($p < 0.05$) among 24 tests was not considered strong evidence for including treatment.

DISCUSSION

In two well-characterized ambulatory trial cohorts, a biomarker panel testing various domains of cardiovascular stress generally showed mild correlations with baseline cardiorespiratory fitness in patients with chronic HFrEF and HFpEF. Most notably, there was only a weak association between change in cardiorespiratory fitness and a change in biomarkers over 4–6 months both in HFrEF and HFpEF. In contrast to NT-proBNP, novel biomarkers demonstrated inconsistent independent associations with cardiorespiratory fitness, suggesting limited incremental utility in the serial monitoring of exercise capacity in HF patients.

The primary purpose of this study was to assess if a select panel of HF biomarkers were associated with CPET performance in patients with HFpEF and HFrEF potentially allowing substitution for cardiorespiratory fitness testing. Developing new tools/biomarkers to predict and track functional capacity is of high importance to patients and providers. The biomarkers we chose to test in this *post-hoc* analysis of two clinical cohorts represent a wide array of surrogates of pathophysiological processes believed to underlie the development and progression of HF. Pathophysiological processes including left ventricular wall stretch from increased volume or pressure (NT-proBNP), inflammation (GDF-15), and cardiovascular stress/fibrosis (ST2 and Galectin 3) represent underlying mechanism and/or disease severity. The three novel biomarkers (GDF-15, ST2 and Galectin 3) in particular were put forward as surrogates of myocardial remodeling given uncovered biological links [21-24]. Irrespective of the mechanistic role of these surrogate markers, all the novel biomarkers have been shown to incrementally improve risk prediction for adverse clinical outcomes such as acute HF and sudden cardiac death in chronic HF often exceeding that of the natriuretic peptides [21, 25, 26]. Some evidence suggests that the same associations may extend to NT-proBNP [15, 16, 27, 28], GDF-15 [29], ST2 [30], Galectin 3 [31, 32] and to measures of cardiorespiratory fitness, which could present a particularly helpful objective tool in the long-term management of chronic HF patients. Our data support prior reports and extend them through the use of a well-phenotyped trial patient population, use of standardized CPET protocols and clinically practical 6MWD and inclusion of HFrEF and HFpEF patients. At baseline peak VO_2 , VE/VCO_2 slope and 6MWD were only mildly correlated with the four biomarkers in HFrEF and HFpEF. When adjusted for all biomarkers, only NT-proBNP and GDF-15 remained independently associated with peak VO_2 and VE/VCO_2 slope, irrespective of HFrEF or HFpEF status.

More importantly, we evaluated whether a change in cardiorespiratory fitness correlated with a change in any of the four tested biomarkers and thus would allow detection of trends in cardiorespiratory fitness. We observed a wide range in functional parameter changes in both trials under investigation. For example, a change in peak VO_2 by 6% (~ 0.7 - 0.8 ml/min^2) from the baseline is considered clinically significant and was observed in the majority of the patients across both trials [9]. There was only a weak to modest correlation between a change in functional parameters and biomarkers across study follow up. In a multivariable analysis, we found no independent association between GDF-15, ST2 or Galectin 3 with any of the tested CPET parameters, despite prior evidence to suggest potential benefit for serial testing of GDF-15 [33], ST2 [34, 35] and Galectin 3 [26] in chronic HF, given their independent prognostic information for left ventricular remodeling, adverse clinical outcomes and correlation with medical treatment. The only significant association between novel biomarkers was between GDF-15 with 6MWD, the least well validated measure of cardiorespiratory fitness in our analysis [36]. Interestingly, only NT-proBNP was independently associated with a change in both peak VO_2 (in HFpEF) and VE/VCO_2 slope (in HFrEF). Our findings support the potential utility for NT-proBNP to assess improvement or deterioration of cardiorespiratory fitness but do not support the serial use of novel biomarkers for the longitudinal monitoring of exercise capacity in HF patients.

The pathophysiological determinants of cardiorespiratory fitness such as cardiovascular congestion, left ventricular remodeling driven by macrovascular/microvascular ischemia,

fibrosis and inflammation [5] can be captured by a multimodal biomarker profile. However, there appears to be only a limited independent value for most of the novel biomarkers when adjusted for traditional clinical, biochemical parameters and other biomarkers. NT-proBNP appears to stand out when compared to the novel biomarkers, which could suggest that mid-term changes in cardiorespiratory fitness are predominately the result of hemodynamic or congestion-related changes that might be best reflected by NT-proBNP. Various dimensions of ventricular remodeling represented by GDF-15, ST2 and Galectin 3, while good surrogates of overall disease severity, might not be equally well suited to track cardiorespiratory fitness. Further, unlike NT-proBNP, GDF-15, ST2 and Galectin 3 are not cardiac-specific, but merely (over-)expressed in the heart as the result of the HF disease process [37-39]. Thus, the potential contribution of other non-cardiac processes to levels of novel biomarkers may make them less sensitive to changes in cardiac status.

Limitations

This is a *post-hoc* analysis of two randomized, controlled, double-blinded trials, was limited to 16/24 weeks follow-up time, and the studies were not powered to detect changes in the cardiorespiratory fitness endpoints. The two trial populations are not directly comparable. Further, follow-up time and degree of baseline cardiorespiratory impairment varied between cohorts, and so this aspect of the analysis is also not comparable between the two cohorts. These results may not be generalizable to all ambulatory HFrEF and HFpEF phenotypes, especially given that the sample was skewed towards white participants. Lastly, this study must be interpreted in the context of patients with complete biomarkers and cardiorespiratory fitness data at baseline and follow-up, excluding patients with interval death or lost-to-follow-up who may have had greatest disease severity.

Conclusions

In these ambulatory trial cohorts, NT-proBNP was associated with baseline cardiorespiratory fitness in HFrEF and HFpEF and change in cardiorespiratory fitness in HFrEF and HFpEF. In contrast, the novel biomarkers were only modestly associated with the cardiorespiratory fitness but after adjustment for traditional risk factors and NT-proBNP there was no consistent independent association with the outcomes of interest. This suggests that there was only limited utility in using these biomarkers in the serial monitoring of exercise capacity in HF patients beyond what's already available.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: The research reported in this publication was supported by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH) under award number U10 HL084904 (for the Coordinating Center) and award numbers U10 HL110297, U10 HL110342, U10 HL110309, U10 HL110262, U10 HL110338, U10 HL110312, U10 HL110302, U10 HL110336, and U10 HL110337 (for the Regional Clinical Centers). Database management and statistical analyses were performed by the Duke Clinical Research Institute (Durham, NC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH/NHLBI.

Conflict of Interest: M.F. is supported by an American Heart Association Grant, 17MCPRP33460225 and NIH T32 grant 5T32HL007101. S.J.G. is supported by the NIH T32 postdoctoral training grant (5T32HL069749) and a Heart Failure Society of America/ Emergency Medicine Foundation Acute Heart Failure Young Investigator Award funded by Novartis. M.V. is supported by the NHLBI T32 postdoctoral training grant (T32HL007604). All other authors declare no relevant financial disclosures.

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Figure 1.
CONSORT diagram

Figure 2.
Correlation Between Baseline Peak VO₂ and Baseline Biomarkers by HF Type in HFrEF and HFpEF

Figure 3:

Functional Capacity Change vs. Biomarker Change by Heart Failure Type. (A) HFrEF (IRONOUT Trial), Change from Baseline to 16 Weeks Post-Baseline; (B) HFpEF (RELAX Trial, Change from Baseline to 24 Weeks Post-Baseline

Figure 4.
Multivariable Association of Biomarker Change with Functional Capacity Change by HF Type with Placebo Group Sensitivity Analysis

Central Figure:
Review of Results

Table 1.

Baseline characteristics in the two trial cohorts

Characteristic	HFrEF (IRONOUT Trial) (N=225)	HFpEF (RELAX Trial) (N=216)
Demographics		
Age, years: median (Q1, Q3) [N]	63 (55, 70) [225]	69 (62, 77) [216]
Female sex: n/N (%)	80 / 225 (35.6%)	104 / 216 (48.1%)
Self-reported white race: n/N (%)	164 / 225 (72.9%)	197 / 216 (91.2%)
Clinical Assessment		
Body mass index, kg/m ² : median (Q1, Q3) [N]	29.2 (25.7, 33.8) [224]	32.9 (28.3, 39.1) [216]
Elevated jugular venous pressure ^[1] : n/N (%)	26 / 222 (11.7%)	95 / 209 (45.5%)
Heart rate, beats/min: median (Q1, Q3) [N]	71 (64, 79) [225]	69 (61, 78) [216]
Left ventricular ejection fraction, %: median (Q1, Q3) [N]	25 (20, 34) [225]	60 (56, 65) [213]
New York Heart Association functional classification ^[2] : n/N (%)		
II	149 / 225 (66.2%)	101 / 216 (46.8%)
III	75 / 225 (33.3%)	115 / 216 (53.2%)
Peripheral edema >= trace: n/N (%)	63 / 225 (28.0%)	125 / 216 (57.9%)
Systolic blood pressure, mmHg: median (Q1, Q3) [N]	112 (98, 125) [225]	126 (113, 138) [216]
Medical History		
Atrial fibrillation/flutter ^[3] : n/N (%)	86 / 222 (38.7%)	111 / 216 (51.4%)
Chronic kidney disease >= stage 3: n/N (%)	124 / 225 (55.1%)	119 / 216 (55.1%)
Diabetes mellitus: n/N (%)	88 / 225 (39.1%)	93 / 216 (43.1%)
HF hospitalization in past year: n/N (%)	97 / 225 (43.1%)	79 / 216 (36.6%)
Hypertension: n/N (%)	162 / 224 (72.3%)	183 / 216 (84.7%)
Ischemic heart disease: n/N (%)	175 / 225 (77.8%)	84 / 216 (38.9%)
Medications at Enrollment		
Mineralocorticoid receptor antagonist: n/N (%)	136 / 225 (60.4%)	23 / 216 (10.6%)
Angiotensin converting enzyme inhibitor or angiotensin II receptor blocker: n/N (%)	189 / 225 (84.0%)	152 / 216 (70.4%)
Beta-blocker: n/N (%)	216 / 225 (96.0%)	164 / 216 (75.9%)
Loop diuretic: n/N (%)	185 / 224 (82.6%)	166 / 216 (76.9%)
Laboratory Results		
Blood urea nitrogen, mg/dL: median (Q1, Q3) [N]	22 (16, 30) [223]	25 (17, 33) [176]
Creatinine, mg/dL: median (Q1, Q3) [N]	1.2 (1.0, 1.5) [225]	1.2 (0.9, 1.5) [216]
Cystatin C, mg/L: median (Q1, Q3) [N]	1.1 (0.8, 1.3) [223]	1.3 (1.1, 1.7) [214]
Hemoglobin, g/dL: median (Q1, Q3) [N]	12.6 (11.8, 13.4) [224]	12.9 (11.9, 13.8) [215]

^[1]Recorded as elevated/distended in IRONOUT, and as >= 8 cm H₂O in RELAX.

^[2]One patient was classified as having class I symptoms in IRONOUT.

^[3]Recorded as atrial fibrillation in IRONOUT, and as atrial fibrillation/flutter in RELAX.

Table 2.

Biomarker Profile and Functional Parameters in HFrEF and HFpEF Ambulatory Patients

Characteristic	HFrEF (IRONOUT Trial) (N=225)	HFpEF (RELAX Trial) (N=216)
Laboratory Results *		
Galectin-3, ng/mL: median (Q1, Q3) [N]	14 (11, 18) [196]	14 (11, 18) [208]
GDF15, pg/mL: median (Q1, Q3) [N]	1331 (875, 2046) [201]	2324 (1537, 3713) [161]
NT-proBNP, pg/mL: median (Q1, Q3) [N]	1111 (453, 2412) [222]	700 (283, 1553) [213]
ST2, ng/mL: median (Q1, Q3) [N]	28 (22, 38) [204]	34 (27, 47) [174]
CPET / Functional Capacity		
Peak VO ₂ , mL/min/kg: median (Q1, Q3) [N]	13.2 (11.1, 15.7) [224]	11.7 (10.2, 14.4) [215]
Peak respiratory exchange ratio: median (Q1, Q3) [N]	1.13 (1.06, 1.20) [224]	1.09 (1.02, 1.15) [215]
VE/VCO ₂ Slope: median (Q1, Q3) [N]	34 (30, 40) [224]	33 (28, 38) [210]
Six-minute walk distance, m: median (Q1, Q3) [N]	363 (292, 428) [225]	308 (229, 383) [216]

* Reference values: Galectin-3 < 22.1 ng/mL; GDF-15 ≤ 750 pg/mL; NT-proBNP < 300pg/mL; ST-2 ≤ 35ng/mL

Table 3.

Multivariable Association of Baseline Biomarkers with Baseline Functional Capacity by HF Type

		Adjusted ^[1]	
Outcome	Biomarker	Mean Difference (95% CI) ^[2]	P-value
HFrEF (IRONOUT Trial)			
Peak VO ₂ , mL/kg/min	Log ₂ (Galectin-3), per doubling	0.291 (−0.545, 1.126)	0.495
	Log ₂ (GDF15), per doubling	−1.528 (−2.179, −0.876)	<.001
	Log ₂ (NT-proBNP), per doubling	−0.344 (−0.585, −0.103)	0.005
	Log ₂ (ST2), per doubling	−0.794 (−1.619, 0.030)	0.059
VE/VCO ₂ slope, % relative difference	Log ₂ (Galectin-3), per doubling	−2.959 (−8.897, 3.366)	0.351
	Log ₂ (GDF15), per doubling	5.370 (0.244, 10.759)	0.040
	Log ₂ (NT-proBNP), per doubling	3.398 (1.501, 5.330)	< .001
	Log ₂ (ST2), per doubling	3.347 (−2.998, 10.107)	0.308
Six-minute walk distance, m	Log ₂ (Galectin-3), per doubling	−7.292 (−38.253, 23.669)	0.644
	Log ₂ (GDF15), per doubling	−33.579 (−57.645, −9.513)	0.006
	Log ₂ (NT-proBNP), per doubling	−2.322 (−11.099, 6.454)	0.604
	Log ₂ (ST2), per doubling	−10.228 (−40.492, 20.036)	0.508
HFpEF (RELAX Trial)			
Peak VO ₂ , mL/kg/min	Log ₂ (Galectin-3), per doubling	−0.033 (−0.695, 0.629)	0.922
	Log ₂ (GDF15), per doubling	−1.010 (−1.487, −0.532)	<.001
	Log ₂ (NT-proBNP), per doubling	−0.464 (−0.676, −0.251)	<.001
	Log ₂ (ST2), per doubling	0.341 (−0.343, 1.024)	0.329
VE/VCO ₂ slope, % relative difference	Log ₂ (Galectin-3), per doubling	−1.265 (−6.846, 4.650)	0.668
	Log ₂ (GDF15), per doubling	4.438 (−0.078, 9.158)	0.054
	Log ₂ (NT-proBNP), per doubling	2.523 (0.653, 4.427)	0.008
	Log ₂ (ST2), per doubling	1.557 (−3.969, 7.400)	0.588
Six-minute walk distance, m	Log ₂ (Galectin-3), per doubling	2.626 (−23.743, 28.995)	0.845
	Log ₂ (GDF15), per doubling	−4.407 (−23.284, 14.470)	0.647
	Log ₂ (NT-proBNP), per doubling	−4.555 (−12.804, 3.693)	0.279
	Log ₂ (ST2), per doubling	−24.963 (−49.844, −0.083)	0.049

^[1] Adjusted for age, sex, body mass index, prior HF hospitalization in past year, systolic blood pressure, blood urea nitrogen, site, and all other biomarkers.

^[2] Estimated difference in given units of outcome per given unit difference in biomarker.

Table 4.

Multivariable Association of Biomarker Change with Functional Capacity Change by HF Type

		Adjusted ^[1]	
Outcome	Biomarker	Mean Difference (95% CI) ^[2]	P-value
HFrEF (IRONOUT Trial), Change from Baseline to 16 Weeks Post-Baseline			
Peak VO ₂ , mL/kg/min	Log ₂ (Galectin-3), per doubling	0.501 (−0.572, 1.575)	0.360
	Log ₂ (GDF15), per doubling	−0.195 (−1.176, 0.785)	0.696
	Log ₂ (NT-proBNP), per doubling	−0.206 (−0.573, 0.161)	0.271
	Log ₂ (ST2), per doubling	−0.954 (−1.938, 0.030)	0.057
VE/VCO ₂ slope, % relative difference	Log ₂ (Galectin-3), per doubling	6.461 (−2.257, 15.956)	0.151
	Log ₂ (GDF15), per doubling	0.175 (−7.119, 8.041)	0.964
	Log ₂ (NT-proBNP), per doubling	3.596 (0.779, 6.492)	0.012
	Log ₂ (ST2), per doubling	4.578 (−2.929, 12.667)	0.239
Six-minute walk distance, m	Log ₂ (Galectin-3), per doubling	−8.112 (−43.901, 27.676)	0.657
	Log ₂ (GDF15), per doubling	−27.096 (−58.850, 4.658)	0.094
	Log ₂ (NT-proBNP), per doubling	−2.187 (−14.490, 10.117)	0.728
	Log ₂ (ST2), per doubling	−3.443 (−36.437, 29.552)	0.838
HFpEF (RELAX Trial), Change from Baseline to 24 Weeks Post-Baseline			
Peak VO ₂ , mL/kg/min	Log ₂ (Galectin-3), per doubling	0.193 (−0.516, 0.903)	0.593
	Log ₂ (GDF15), per doubling	−0.599 (−1.369, 0.171)	0.127
	Log ₂ (NT-proBNP), per doubling	−0.726 (−1.100, −0.353)	< .001
	Log ₂ (ST2), per doubling	−0.123 (−1.100, 0.854)	0.805
VE/VCO ₂ slope, % relative difference	Log ₂ (Galectin-3), per doubling	−3.942 (−9.097, 1.505)	0.153
	Log ₂ (GDF15), per doubling	2.756 (−2.991, 8.843)	0.355
	Log ₂ (NT-proBNP), per doubling	2.922 (−0.048, 5.980)	0.054
	Log ₂ (ST2), per doubling	−0.294 (−7.394, 7.351)	0.938
Six-minute walk distance, m	Log ₂ (Galectin-3), per doubling	−17.050 (−45.540, 11.440)	0.241
	Log ₂ (GDF15), per doubling	−31.606 (−61.404, −1.809)	0.038
	Log ₂ (NT-proBNP), per doubling	−10.783 (−25.518, 3.953)	0.152
	Log ₂ (ST2), per doubling	1.492 (−34.373, 37.357)	0.935

^[1] Adjusted for age, sex, body mass index, prior HF hospitalization in past year, systolic blood pressure, blood urea nitrogen, site, baseline biomarker levels, baseline outcome, and all other biomarkers.

^[2] Estimated change in given units of outcome from baseline to 16 (24) weeks per given unit change in biomarker from baseline to 16 (24) weeks.