Galectin-3 in Heart Failure With Preserved Ejection Fraction
A RELAX Trial Substudy (Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Diastolic Heart Failure)

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ABSTRACT

OBJECTIVES This study hypothesized that elevated galectin-3 (Gal-3) levels would identify patients with more advanced heart failure (HF) with preserved ejection fraction (HFpEF) as assessed by key pathophysiological domains.

BACKGROUND Gal-3 is implicated in the pathogenesis of cardiac fibrosis but is also increased with normal aging and renal dysfunction. Cardiac fibrosis may contribute to cardiac dysfunction, exercise intolerance, and congestion in HFpEF.

METHODS Two hundred eight patients from the RELAX (Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Diastolic Heart Failure) trial of sildenafil in HFpEF had Gal-3 measured at enrollment. Pathophysiological domains assessed included biomarkers of neurohumoral activation, fibrosis, inflammation and myocardial necrosis, congestion severity and quality of life, cardiac structure and function, and exercise performance. Analysis adjusted for age, sex, and/or cystatin-C levels. Potential interaction between baseline Gal-3 and treatment (sildenafil) effect on the RELAX study primary endpoint (change in peak oxygen consumption) was tested.

RESULTS Gal-3 levels were associated with age and severity of renal dysfunction. Adjusting for age, sex, and/or cystatin-C, Gal-3 was not associated with biomarkers of neurohumoral activation, fibrosis, inflammation or myocardial necrosis, congestion or quality-of-life impairment, cardiac remodeling or dysfunction, or exercise intolerance. Gal-3 did not identify patients who responded to phosphodiesterase type 5 (PDE-5) inhibitors (interaction p = 0.53).

CONCLUSIONS In overt HFpEF, Gal-3 was related to severity of renal dysfunction and accounting for this, was not independently associated with severity of pathophysiological derangements or response PDE-5 inhibition. These findings underscore the need to adjust for renal function when interpreting Gal-3 levels, and call into question the value of Gal-3 to quantify disease severity in overt HFpEF. (J Am Coll Cardiol HF 2015;3:245–52) © 2015 by the American College of Cardiology Foundation.
Approximately 50% of patients with chronic heart failure (HF) have preserved ejection fraction (HFpEF) (1). Coronary microvascular endothelial and myocardial inflammation may play a role in the genesis of cardiac fibrosis in HFpEF (2). Galectin-3 (Gal-3) is secreted by activated macrophages and has been implicated in the regulation of pro-inflammatory and profibrotic pathways in the heart (3–6). In rodent models, myocardial Gal-3 expression predicts future HF (7), exogenous Gal-3 administration promotes fibrosis and HF (4), and genetic or pharmacologic inhibition of Gal-3 attenuates fibrosis and cardiac dysfunction in response to pro-fibrotic stimuli (6,8).

Given the role of myocardial inflammation and fibrosis in the pathogenesis of HFpEF, Gal-3 may serve as a novel biomarker of HFpEF severity that is incremental to established biomarkers and readily available clinical information such as renal function (9,10). In this regard, studies of persons without HF (11,12) and of patients with HF with reduced ejection fraction (HFrEF) (13–17) have shown that Gal-3 levels are associated with the severity of renal dysfunction.

Although circulating levels of Gal-3 have been shown to be associated with outcomes (13–15,17–21), exercise intolerance (13,21), and treatment effect of statins (22) and angiotensin receptor blockers (17) in HFrEF, data regarding the relationship of Gal-3 levels to renal function, markers of HF severity, or treatment response in HFpEF are lacking.

The RELAX (Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Diastolic Heart Failure) trial tested the effect of sildenafil on exercise capacity in 216 well-characterized patients with HFpEF. We hypothesized that higher levels of Gal-3 would be associated with worse HF as evidenced by more severe derangements in biomarkers of neurohumoral activation, fibrosis, inflammation and myocardial necrosis, cardiac structure and function, exercise capacity, congestion and quality of life. Finally, although the RELAX trial showed no effect of sildenafil on exercise capacity in HFpEF, we hypothesized that higher Gal-3 levels might identify patients with more advanced myocardial derangements, pulmonary hypertension, and right ventricular (RV) dysfunction, a subset postulated to be sensitive to sildenafil (23). Thus, we investigated the potential for interaction between Gal-3 levels and treatment effect of sildenafil.

**METHODS**

The RELAX trial was conducted by the Heart Failure Clinical Research Network (HFN) and funded by the National Heart, Lung, and Blood Institute (24). All patients provided written informed consent and the trial was approved by the institutional review board at each participating site.

The design, entry criteria and results of the RELAX trial have been reported previously (24,25). Briefly, the RELAX trial enrolled 216 outpatients who had ejection fraction ≥50% and objective evidence of HF. Additionally, patients 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 peak oxygen consumption [V̇O₂peak]). Patients with an estimated glomerular filtration rate (GFR) (Modification of Diet in Renal Disease equation) <20 ml/min/1.73 m² were ineligible.

Participants underwent baseline studies, which included a history and physical examination, echocardiography, cardiac magnetic resonance imaging (CMRI) if in sinus rhythm (n = 115), cardiopulmonary exercise test (CPXT), 6-min walk test, Minnesota Living with Heart Failure Questionnaire, and phlebotomy for biomarkers (25).

Comprehensive Doppler echocardiography and CMRI were performed according to study protocols with measurements performed at the HFN echocardiography (Mayo Clinic, Rochester, Minnesota) and CMRI (Duke University, Durham, North Carolina) core laboratories. CPXT was performed according to a RELAX-specific protocol and interpreted by the HFN CPXT core laboratory (Massachusetts General Hospital, Boston, Massachusetts) as previously reported (24,25). Plasma biomarker measurements were performed by the HFN biomarker core laboratory (University of Vermont, Burlington, Vermont) as previously described (24,25) and included markers of neurohumoral activation (NT-proBNP, aldosterone, endothelin-1), renal function (cystatin-C, creatinine, uric acid), fibrosis (Gal-3, pro-collagen III N-terminal peptide, C-telopeptide for type I collagen [CITP]), and myocardial injury or inflammation (high-sensitivity troponin-I and high-sensitivity C-reactive protein).

Gal-3 levels were measured by enzyme-linked immunosorbent assay (cat #DGAL30, R&D Systems, Minneapolis, Minnesota). The manufacturer’s healthy reference listed a mean of 6.44 ng/ml, with a range of 2.03 to 15.5 ng/ml and a SD of 2.1 ng/ml. This assay compared well with another widely used assay...
BG Medicine ELISA, Waltham, Massachusetts) when tested in 70 RELAX trial patients (Gal-3_{BG} = 1.08 \times Gal-3_{R&D} + 3.61; Pearson R = 0.92).

**STATISTICAL ANALYSIS.** Data are presented as medians (interquartile ranges) or proportions across Gal-3 tertiles. Differences across Gal-3 tertiles were tested with Kruskal-Wallis, chi-square, or Fisher exact tests, as appropriate. Multivariable least-squares linear regression was used to adjust for age, sex, and cystatin-C, and for cystatin-C alone. These variables were chosen given their previously reported association with Gal-3 levels in persons without HF (11,12). Association between Gal-3 levels and variables of interest were analyzed with Gal-3 as a categorical variable (tertiles) and in a sensitivity analysis, as a continuous (log transformed) variable. Relationships of cystatin-C and GFR with Gal-3 are shown in scatterplots with regression lines and Pearson correlation coefficients. A general linear model adjusting for treatment group, baseline peak VO₂, and Gal-3 levels was used to examine interaction between treatment group and Gal-3 levels on change in peak VO₂ from baseline to 24 weeks. With our sample size, we had 80% and 90% power to detect correlations of 0.195 and 0.225, respectively, between Gal-3 and other continuous measures. Analyses were performed by the HFN data coordinating center using SAS version 9.2 (SAS Institute, Cary, North Carolina). A p < 0.05 (2-sided) was considered statistically significant.

**RESULTS**

Of the 216 participants enrolled in the RELAX trial, 208 had Gal-3 level data at baseline and comprise the study group. As previously reported, overall, patients in RELAX had a median age of 69 years, 52% were men, and comorbidities (hypertension, diabetes, atrial fibrillation, coronary artery disease) were common (24). The median ejection fraction was 60%, and participants were commonly treated with cardiovascular medications, with 71% on angiotensin-converting enzyme inhibitors and 11% on aldosterone antagonists. Median GFR was 64 ml/min/1.73 m². The median Gal-3 level was 13.8 ng/ml, with a range from 4.2 to 41.6 ng/ml (Figure 1).

**CLINICAL CHARACTERISTICS AND GAL-3 LEVELS IN HFpEF.** Participants with higher Gal-3 levels were older, had a lower body surface area, and were more likely to have diabetes and be treated with mineralocorticoid receptor antagonists and diuretics (Table 1). Patients with higher Gal-3 levels had worse renal function (Table 1, Figure 2) as assessed by creatinine, estimated GFR, or cystatin-C, and expected sequelae of renal dysfunction including lower hemoglobin and higher uric acid levels. Age, sex, and cystatin-C levels explained 24% of the variability in Gal-3 levels in HFpEF. Cystatin-C alone explained 23% of the variability in Gal-3 levels. Adjusting for age, sex, and/or cystatin-C, patients with higher Gal-3 levels had lower body surface area, but there were no other statistically significant associations with clinical characteristics, medication use, or likelihood of previous hospitalization.

**BIOMARKERS AND GAL-3 LEVELS IN HFpEF.** NT-proBNP and endothelin-1, but not aldosterone, levels were higher in patients with higher Gal-3 levels in bivariate analysis, but not after adjusting for age, sex, and/or cystatin-C (Table 1). Similarly, Gal-3 levels were associated with fibrosis biomarkers (procollagen III N-terminal peptide and CITP), but not after adjustment for age, sex, and/or cystatin-C. Gal-3 was not correlated with high-sensitivity C-reactive protein. Subjects with high Gal-3 levels tended to have higher high-sensitivity troponin I levels, but not after adjusting for age, sex, and/or cystatin-C.

**SYMPTOMS AND CONGESTION AND GAL-3 LEVELS IN HFpEF.** Gal-3 levels were not associated with symptom severity as assessed by New York Heart Association functional class or Minnesota Living with Heart Failure Questionnaire score (Table 2). Gal-3 appeared to be modestly associated with congestion as patients with higher Gal-3 levels had higher NT-proBNP levels (Table 1), a higher prevalence of orthopnea and a trend towards more peripheral

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**Tables and Figures:**

- **Table 1:** Clinical characteristics and Gal-3 levels in HFpEF.
- **Table 2:** Symptoms and congestion and Gal-3 levels in HFpEF.
- **Figure 1:** Frequency distribution of Gal-3 levels in HFpEF.
edema (Table 2), but not after adjustment for age, sex, and/or cystatin-C. There was no association between Gal-3 and jugular venous pressure elevation or rises on physical examination.

**CARDIOVASCULAR STRUCTURE AND FUNCTION AND GAL-3 LEVELS IN HFpEF.** Subjects with higher Gal-3 levels had greater wall thickness by echocardiography, but not after adjusting for age, sex, and/or cystatin-C (Table 3). There was no similar trend in the CMRI cohort (left ventricular [LV] mass/LV volume ratio). Neither body size-indexed LV diastolic dimension or mass, ejection fraction, LV diastolic function parameters, nor pulmonary artery systolic pressure differed across Gal-3 tertiles by echocardiography or CMRI in unadjusted analysis or when adjusting for age, sex, and cystatin-C.

There was no association between systolic blood pressure and Gal-3 levels. Subjects with higher Gal-3 levels displayed lower diastolic blood pressure, but not after adjusting for age, sex, and/or cystatin-C (Table 3). In the CMRI subset, adjusting for age, sex, and cystatin-C, aortic distensibility was lower in subjects with higher Gal-3 levels, but this measurement was only available in a small subset of patients.

**EXERCISE PERFORMANCE AND GAL-3 LEVELS IN HFpEF.** Patients with higher Gal-3 levels had lower peak VO₂ despite similar effort (respiratory exchange ratio) and peak exercise systolic blood pressure (Table 4). Patients with higher Gal-3 levels also had lower peak heart rate and chronotropic index. Six-min walk distance was lower in patients with higher Gal-3 levels. However, when adjusted for age, sex,
and/or cystatin-C, associations between Gal-3 levels and exercise performance and chronotropic reserve were no longer significant. There was also no association between Gal-3 levels and peak VO2 when adjusting for known modifiers of peak VO2 (age, sex, body mass index, hemoglobin, and chronotropic index; \( p = 0.42 \)). There was no association between Gal-3 levels and ventilatory efficiency (VE/VCO2 slope).

**SENSITIVITY ANALYSIS.** Findings were similar when analyses were performed with Gal-3 as a continuous, log-transformed variable (data not shown).

**GAL-3 LEVELS AS A BIOMARKER OF RESPONSE TO SILDENAFIL.** There was no interaction between baseline Gal-3 and treatment group (sildenafil vs. placebo) on the RELAX trial primary endpoint of change in peak VO2 after 6 months of therapy (p value for interaction = 0.53).

**DISCUSSION**

In keeping with recent recommendations (9,10), this robust analysis assessed the performance of Gal-3 as an independent biomarker of HFpEF severity adjusting for readily available information. In this comprehensively phenotyped cohort of HFpEF patients, Gal-3 levels were associated with age, smaller body size, and severity of renal dysfunction. Adjusting for age, sex, and cystatin-C or cystatin-C alone, Gal-3 levels were not associated with clinical characteristics or comorbidities, symptomatic status or congestion, severity of LV remodeling or dysfunction, or exercise performance. Patients with higher Gal-3 levels had more neurohumoral activation and higher levels of fibrosis biomarkers, but no evidence of increased inflammation, and these associations were largely eliminated after adjustment for age, sex, and/or cystatin-C. The absence of treatment effect (sildenafil vs. placebo) on exercise capacity in the RELAX trial was consistent among patients regardless of Gal-3 levels. Our negative findings are additive to the scant available published reports concerning Gal-3 in HFpEF and do not suggest that Gal-3 levels reflect disease severity in overt HFpEF.

**GAL-3 AS A BIOMARKER IN HFPEF.** The median level of Gal-3 in the ambulatory HFpEF cohort enrolled in RELAX was more than twice the assay-specific normal level and similar to (13,15) or slightly lower (17,27) than mean levels reported in various HFrEF cohorts. Many previous studies used the BG assay. Given the relationship between the 2 assays, Gal-3 levels would be 3 to 5 ng/ml higher in the RELAX trial had the BG assay been used. Thus, plasma Gal-3 levels in the RELAX trial were similar to or higher than those observed in HFrEF cohorts.

**Association of Gal-3 levels with renal function.** As observed here in HFpEF, community cross-sectional

| TABLE 2: Congestion and Quality of Life by Tertiles of Baseline Gal-3 Levels |
|-----------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                 | Low Gal-3 Tertile (n = 69) | Mid Gal-3 Tertile (n = 70) | High Gal-3 Tertile (n = 69) | p Value | p Value* | p Value† |
| NYHA functional class II | 37 (54) | 31 (44) | 29 (42) | 0.35 | 0.77 | 0.74 |
| MLHFQ score (n = 200) | 44 (31-66) | 46 (32-61) | 40 (25-57) | 0.30 | 0.80 | 0.66 |
| Elevated JVP (n = 202) | 28 (42) | 30 (44) | 33 (49) | 0.69 | 0.80 | 0.91 |
| Rales present | 2 (3) | 5 (7) | 7 (10) | 0.23 | 0.53 | 0.60 |
| S3 present | 3 (4) | 3 (4) | 2 (3) | 1.00 | 0.94 | 0.95 |
| Moderate edema | 6 (9) | 15 (21) | 20 (29) | 0.01 | 0.12 | 0.12 |
| Orthopnea (n = 194) | 34 (53) | 41 (64) | 47 (71) | 0.10 | 0.35 | 0.43 |

Values are n (%) or median (interquartile range). *Adjusted for age, sex, cystatin-C. †Adjusted for cystatin-C alone. Data available in all 208 participants except as noted.

Gal-3 = galectin-3; JVP = jugular venous pressure; MLHQF = Minnesota Living with Heart Failure Questionnaire; NYHA = New York Heart Association; S3 = S3 gallop.
studies indicate that Gal-3 levels increase with age and renal dysfunction, and also show that Gal-3 levels are higher in women than in men (11,12). Our study did not include an age-, sex-, and cystatin-C-matched non-HF cohort, so the increase in Gal-3 conferred by the HFpEF state (vs. renal dysfunction) cannot be assessed. Gopal et al. (16) studied acutely decompensated or stable HFrEF or HFpEF patients, chronic kidney disease patients without HF, and normal controls, and reported that Gal-3 levels increase exponentially with the severity of renal dysfunction and that this relationship is similar regardless of presence, type, or severity of HF. In the RELAX trial, the relationship between renal dysfunction and Gal-3 levels was linear and although strong, weaker than that reported in the Gopal et al. study, which included patients with GFR <20 and noted an exponential relationship driven by extremely high Gal-3 levels in patients with GFR <20 (excluded in the RELAX trial).

### Table 3: Baseline Cardiac Structure and Function by Tertiles of Baseline Gal-3 Levels

<table>
<thead>
<tr>
<th>Echocardiography</th>
<th>N</th>
<th>Low Gal-3 Tertile (n = 69)</th>
<th>Mid Gal-3 Tertile (n = 70)</th>
<th>High Gal-3 Tertile (n = 69)</th>
<th>p Value</th>
<th>p Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDV/BSA, cm/m²</td>
<td>157</td>
<td>2.2 (2.1-2.5)</td>
<td>2.2 (2.0-2.4)</td>
<td>2.2 (2.0-2.4)</td>
<td>0.96</td>
<td>0.96</td>
<td>0.98</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>151</td>
<td>0.38 (0.35-0.44)</td>
<td>0.40 (0.34-0.51)</td>
<td>0.45 (0.40-0.52)</td>
<td>0.006</td>
<td>0.71</td>
<td>0.73</td>
</tr>
<tr>
<td>LV mass/BSA, g/m²</td>
<td>151</td>
<td>75 (63-88)</td>
<td>77 (63-91)</td>
<td>80 (60-102)</td>
<td>0.78</td>
<td>0.97</td>
<td>0.93</td>
</tr>
<tr>
<td>LV mass/hectare¹, g/m²¹</td>
<td>151</td>
<td>63 (52-76)</td>
<td>67 (54-84)</td>
<td>66 (54-88)</td>
<td>0.64</td>
<td>0.71</td>
<td>0.85</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>205</td>
<td>61 (57-65)</td>
<td>60 (55-67)</td>
<td>60 (56-65)</td>
<td>0.47</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>137</td>
<td>1.2 (1.0-2.0)</td>
<td>1.5 (1.0-2.0)</td>
<td>1.3 (0.9-2.0)</td>
<td>0.55</td>
<td>0.23</td>
<td>0.34</td>
</tr>
<tr>
<td>Medial e’, m/s</td>
<td>190</td>
<td>0.06 (0.05-0.07)</td>
<td>0.06 (0.05-0.09)</td>
<td>0.06 (0.04-0.08)</td>
<td>0.22</td>
<td>0.13</td>
<td>0.20</td>
</tr>
<tr>
<td>Medial E/e’</td>
<td>181</td>
<td>14.6 (10.0-20.0)</td>
<td>15.7 (12.0-22.3)</td>
<td>17.5 (13.3-25.0)</td>
<td>0.17</td>
<td>0.99</td>
<td>0.94</td>
</tr>
<tr>
<td>Deceleration time, ms</td>
<td>186</td>
<td>181 (155-219)</td>
<td>190 (158-215)</td>
<td>183 (153-220)</td>
<td>0.95</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>LA volume/BSA, ml/m²</td>
<td>144</td>
<td>43 (33-59)</td>
<td>42 (34-58)</td>
<td>46 (38-59)</td>
<td>0.48</td>
<td>0.59</td>
<td>0.84</td>
</tr>
<tr>
<td>PASP, mm Hg</td>
<td>131</td>
<td>41 (35-54)</td>
<td>37 (30-49)</td>
<td>45 (35-51)</td>
<td>0.19</td>
<td>0.24</td>
<td>0.26</td>
</tr>
</tbody>
</table>

### Table 4: Exercise Performance by Tertile of Baseline Gal-3 Levels

<table>
<thead>
<tr>
<th>Echocardiography</th>
<th>N</th>
<th>Low Gal-3 Tertile (n = 69)</th>
<th>Mid Gal-3 Tertile (n = 70)</th>
<th>High Gal-3 Tertile (n = 69)</th>
<th>p Value</th>
<th>p Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak VO₂, ml/kg/min</td>
<td>13.3 (10.8-16.2)</td>
<td>11.7 (10.9-13.7)</td>
<td>11.1 (9.5-13.7)</td>
<td>0.004</td>
<td>0.68</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>1.11 (1.03-1.18)</td>
<td>1.08 (1.02-1.14)</td>
<td>1.09 (1.04-1.15)</td>
<td>0.40</td>
<td>0.50</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Peak systolic BP, mm Hg</td>
<td>158 (140-174)</td>
<td>157 (133-173)</td>
<td>148 (129-166)</td>
<td>0.19</td>
<td>0.67</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Rest HR, beats/min</td>
<td>70.0 (61.5-77.0)</td>
<td>70 (60-80)</td>
<td>65 (59-75)</td>
<td>0.17</td>
<td>0.46</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Peak HR, beats/min</td>
<td>114 (101-133)</td>
<td>111 (93-128)</td>
<td>97 (86-118)</td>
<td>0.003</td>
<td>0.55</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Chronotropic index</td>
<td>0.57 (0.36-0.69)</td>
<td>0.53 (0.32-0.71)</td>
<td>0.37 (0.25-0.61)</td>
<td>0.034</td>
<td>0.74</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>VE/VCO₂ slope</td>
<td>32.4 (28.2-37.1)</td>
<td>31.7 (28.5-36.7)</td>
<td>34.1 (28.9-37.9)</td>
<td>0.69</td>
<td>0.92</td>
<td>0.99</td>
<td></td>
</tr>
</tbody>
</table>

Values are median (interquartile range). *Adjusted for age, sex, cystatin-C. †Adjusted for cystatin-C alone. Total N with data = 201 to 208.
The mechanism underlying the association between renal impairment and Gal-3 levels is unclear and could include renal production or clearance of Gal-3 or covariance mediated by conditions common to or causative of renal impairment and HF (16). Of note, myocardial Gal-3 levels were up-regulated in cardiac tissue from patients with aortic stenosis associated with systolic dysfunction (4), but not in myocardial specimens from patients with HFrEF, despite elevated plasma Gal-3 levels (28,29), and Gal-3 levels do not decrease post-total artificial heart implantation (30). Thus, the source of elevated Gal-3 levels in HF is unclear and may be predominantly noncardiac.

**Association of Gal-3 levels with cardiac structure and function.** Although an association between Gal-3 and ventricular dysfunction was demonstrated in animal studies (6), a relationship between circulating Gal-3 levels and the severity of cardiac remodeling or dysfunction in human studies is less clear (11,15,31). In a cross-sectional volunteer cohort, Gal-3 levels were modestly associated with LV mass, but this analysis did not adjust for renal function (11). In a diverse cohort of patients evaluated in an emergency department for dyspnea and subsequently found to have HFrEF, HFpEF, or noncardiac dyspnea (31), Gal-3 levels were higher in HF than noncardiac dyspnea. In this analysis including patients with and without HF, Gal-3 levels were associated with echocardiographic indices of LV diastolic dysfunction, RV systolic dysfunction, and pulmonary artery pressures, but this analysis did not adjust for age, sex or renal function or examine the association of Gal-3 and cardiac parameters within HF cohorts. In a study of HFrEF patients, Gal-3 levels were not associated with echocardiographic parameters or invasively measured hemodynamic indices (15). In the RELAX trial, concentric remodeling was more severe in patients with higher Gal-3 levels, but there was no association between Gal-3 levels and the severity of LV hypertrophy, systolic or diastolic dysfunction, or pulmonary artery systolic pressure, and the modest association with concentric remodeling was no longer apparent after adjusting for age, sex, and/or cystatin-C.

**Association of Gal-3 levels with functional capacity.** In HFpEF patients, we show that higher Gal-3 levels were associated with lower peak \( VO_2 \) and 6-min walk distance but that this association was lost after adjusting for age, sex, and/or cystatin-C. In chronic HFrEF patients, higher Gal-3 levels were associated with poorer functional capacity as assessed by peak \( VO_2 \), 6-min walk distance, or New York Heart Association functional class (13,21), but these studies did not adjust for renal function.

**GAL-3 AS A PREDICTOR OF TREATMENT RESPONSE.** We had hypothesized that higher Gal-3 levels might identify patients with more advanced myocardial derangements, pulmonary hypertension, and RV dysfunction, a subset postulated to be sensitive to sildenafil (23). However, no such associations were apparent, and we saw no interaction between treatment effect and Gal-3 levels.

In contrast to our findings in HFpEF, post-hoc analysis of clinical trials in HFrEF suggest that Gal-3 levels may identify patients responding to certain therapies. Although there was no overall benefit of rosvustatin on outcomes in CORONA (Controlled Rosuvastatin Multinational Trial in HFrEF), a post-hoc subgroup analysis showed those with lower Gal-3 levels appeared to benefit from statin therapy (22). A larger subgroup analysis from the CORONA study showed that patients with lower NT-proBNP levels also benefited from statins (22). Those with lower Gal-3 or NT-proBNP had milder HF, suggesting that both Gal-3 and NT-proBNP identify patients with milder HF who may survive to experience a vascular rather than HF event. In a post-hoc analysis from the Val-HeFT (Valsartan Heart Failure Trial), the incidence of HF hospitalization was lower with valsartan than placebo therapy among patients with lower Gal-3 levels, but no interaction between Gal-3 levels and treatment effect was noted for the trial’s primary endpoint of mortality or first morbid event (17). Milting et al. (30) showed that Gal-3 levels at ventricular assist device implantation were significantly higher in patients who did not survive mechanical support because of multiorgan failure, but did not adjust for baseline renal function, a known risk factor for poor outcomes after LV assist device. Although Gal-3 levels did not identify a group of HFpEF patients who responded to sildenafil, analysis of interaction between treatment effect and Gal-3 levels in clinical trials of other agents in HFpEF, including mineralocorticoid receptor antagonists, will be of interest.

**STUDY LIMITATIONS.** The specific Gal-3 assay used in this study (R&D Systems) differs from previous studies but is well correlated with the BG assay. The RELAX cohort had relatively advanced HFpEF, and this may limit correlations although the range of Gal-3 levels were fairly broad. The association of Gal-3 levels and outcomes was not assessed in the RELAX trial, given the short duration of follow-up and modest sample size. These data do not exclude the possibility that transient myocardial Gal-3 activation occurs in HF and contributes to myocardial fibrosis.
CONCLUSIONS

In HFP EF, Gal-3 levels were associated with higher age and worse renal function, but adjusting for age and renal function, were not independently associated with severity of pathophysiological rearrangements in HFP EF and did not identify patients responding to phosphodiesterase type 5 inhibition. These findings underscore the need to adjust for renal function when interpreting Gal-3 levels and call into question the independent value of Gal-3 to quantify disease severity in overt HFP EF.

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REFERENCES


KEY WORDS biomarkers, diastole, galectin-3, heart failure.