

Relationship of Plasma Galectin-3 to Renal Function in Patients With Heart Failure: Effects of Clinical Status, Pathophysiology of Heart Failure, and Presence or Absence of Heart Failure

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Background—Galectin-3 (GAL-3), a β -galactoside-binding protein, is a new clinical biomarker believed to reflect cardiac remodeling/fibrosis in patients with heart failure (HF). Plasma GAL-3 is inversely related to renal function. It is not known whether the relationship between renal function and GAL-3 is influenced by clinical decompensation, type of HF, or the presence or absence of clinical HF.

Methods and Results—Patients were prospectively categorized as having acute decompensated HF or stable HF on the basis of clinical status and as having HF with reduced left ventricular ejection fraction or HF with preserved left ventricular ejection fraction. Plasma GAL-3 was measured by enzyme-linked immunosorbent assay in patients with HF (n=75), control patients without HF (n=32), and control patients without HF with moderate renal insufficiency (n=12). Compared to controls without HF (14±4 ng/mL), GAL-3 was higher in patients with both acute decompensated HF (23±11 ng/mL) and stable HF (22±10 ng/mL) ($P<0.001$ versus controls for both) but did not differ between acute decompensated HF and stable HF ($P=0.75$). Likewise, GAL-3 was elevated in both HF with preserved left ventricular ejection fraction (23±9 ng/mL) and HF with reduced left ventricular ejection fraction (22±11 ng/mL) ($P<0.001$ versus controls for both) but did not differ between HF with preserved ejection fraction and HF with reduced ejection fraction ($P=0.37$). GAL-3 correlated strongly with estimated glomerular filtration rate, both in patients with HF ($r=-0.75$, $P<0.001$) and in patients without HF ($r=-0.82$, $P<0.001$), and this relationship was unaffected by the presence or absence of clinical HF.

Conclusions—Plasma GAL-3 is inversely related to renal function in patients with and without clinical HF. Concentrations of plasma GAL-3 do not seem to depend on the level of compensation or type of HF. Furthermore, the relationship between GAL-3 and renal function seems to be affected little or not at all by the presence or absence of clinical HF. (*J Am Heart Assoc.* 2012;1:e000760 doi: 10.1161/JAHA.112.000760)

Key Words: biomarkers • galectin-3 • heart failure • kidney

Galectin-3 (GAL-3), a β -galactoside-binding protein secreted by immune cells, recently was approved by the US Food and Drug Administration as a prognostic aid in patients with heart failure (HF).¹ Experimentally, GAL-3 is associated with inflammation and tissue fibrosis,^{2–6} and it has

been associated with myocardial fibrosis, ventricular remodeling, and left ventricular dysfunction.^{7,8} GAL-3 expression is increased in experimental cardiomyopathy.^{8–11} Plasma GAL-3 seems to be a prognostic marker of HF outcomes such as death, recurrent HF, and hospitalization for HF.^{12–14}

Renal function is a recognized determinant of outcomes in HF,^{15,16} and correction for renal impairment has been found to diminish the ability of GAL-3 to predict outcomes in patients with HF in some^{12,17} but not all^{13,14,18} studies. An inverse relationship between GAL-3 and renal function has been observed in patients with HF,^{13,18,19} leading to the suggestion that increased plasma GAL-3 in HF might be due to renal dysfunction, and further, that the ability of GAL-3 to predict outcomes in HF might reflect, at least in part, the consequences of renal impairment. These studies raise questions about the mechanism by which GAL-3 and renal function are related in patients with HF.

Clinical decompensation is associated with impaired renal function,²⁰ as is HF with preserved ejection fraction (HFpEF)

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versus reduced ejection fraction (HFrEF).^{21,22} Accordingly, a goal of the present study was to determine whether increased GAL-3 in HF is related to either the level of compensation or the underlying pathophysiology (ie, HFpEF versus HFrEF). Studies of GAL-3 in HF to date have not included patients with and without renal dysfunction in the absence of HF. Therefore, a second goal was to determine (1) whether the relationship between GAL-3 and renal function is specific to patients with HF or is present in patients with renal dysfunction in the absence of HF, and (2) whether this relationship is affected by the presence of HF. To address these questions, plasma GAL-3 was measured in patients with HF who were prospectively categorized as having decompensated versus stable HF and as having HFpEF versus HFrEF. To assess the relative roles of HF and renal impairment, plasma GAL-3 also was measured in patients who did not have HF but displayed renal functional impairment comparable to the HF groups.

Methods

Study Population

Six groups of patients were prospectively recruited: (1) patients admitted to the hospital for treatment of decompensated HFrEF (n=21), (2) patients admitted to the hospital for treatment of decompensated HFpEF (n=16), (3) ambulatory patients with chronic stable HFrEF (n=20), (4) ambulatory patients with chronic stable HFpEF (n=18), (5) control patients without HF (n=32), and (6) patients without HF but with moderate renal insufficiency due to chronic kidney disease (CKD) (n=12). Patients were assigned to groups on the basis of a review of the medical record and a history and physical examination performed by one of the investigators. The research protocol was approved by the Institutional Review Board at the Boston University Medical Center. Written informed consent was obtained from all participants before enrollment in the study.

Patients with decompensated HF were identified from patients admitted to the hospital for treatment of an episode of HF complicated by volume overload. The diagnosis of decompensated HF was defined clinically as the presence of worsening symptoms of dyspnea, paroxysmal nocturnal dyspnea, or orthopnea in conjunction with clinical signs of circulatory congestion (elevated jugular venous pressure, hepatjugular reflux, hepatomegaly, or peripheral edema). Patients with decompensated HF were enrolled into the study within 24 hours of admission. Patients with chronic stable HF were recruited from the Cardiomyopathy Clinic. These patients had symptoms that had been stable for at least 2 months, had not been hospitalized during the previous 2 months, were not volume overloaded by clinical examination, and did not require a change in diuretic therapy on that

visit. HFrEF was defined as a previous diagnosis of HF and echocardiographic demonstration of left ventricular systolic dysfunction with a left ventricular ejection fraction (LVEF) $\leq 45\%$, whereas HFpEF was defined as an LVEF $\geq 50\%$. Control patients without HF were recruited from the general cardiology ambulatory clinics or inpatient services. None of the control patients without HF had a history of HF or evidence of lung congestion or left ventricular dysfunction at the time of study. An additional 12 patients without HF but with moderate renal insufficiency due to CKD were recruited from an ambulatory nephrology clinic. All urine sediments were bland at the time of enrollment.

Excluded from enrollment were patients with concomitant acute coronary syndromes within the previous 3 months; primary infectious, inflammatory, or rheumatologic processes; hemodynamic instability requiring intravenous vasoactive drugs; aortic stenosis; malignancy; or hepatic dysfunction (cirrhosis or active hepatitis).

Patient Demographics, Risk Factors, and Clinical Laboratory Variables

Patient demographics were recorded upon enrollment. HF risk factors including smoking history, diabetes, cerebrovascular disease, hypertension, and history of ischemic heart disease were determined via clinical chart review or the use of disease-specific medications (ie, antidiabetics or antihypertensive medications). Estimated glomerular filtration rate (eGFR) was determined by the Chronic Kidney Disease Epidemiology Collaboration formula: $eGFR = 141 \times \min(\text{Scr}/k, 1)^a \times \max(\text{Scr}/k, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018$ (if female) $\times 1.159$ (if black).²³

Biomarker Measurements

Blood samples were collected via venipuncture, centrifuged, and then frozen at -70°C until the assays were performed. GAL-3 was analyzed in the plasma with an enzyme-linked immunosorbent assay (BG Medicine, Waltham, MA). N-terminal pro-B-type natriuretic peptide (NT-proBNP) was analyzed in plasma by an enzyme-linked immunosorbent assay (Alpco Diagnostics, Salem, NH). All specimens were processed in duplicate, and the mean intra-assay coefficient of variation was $<7\%$.

Statistical Analysis

Descriptive statistics are presented as mean \pm standard deviation for continuous variables and as percentages for categorical variables. Comparisons among all groups for baseline clinical variables were performed with the Kruskal-Wallis test for continuous variables (as these variables

exhibited a non-normal distribution with normality testing) and with the Pearson χ^2 or Fisher exact test for categorical variables. The Tukey multiple-comparisons test was used for testing all other multiple comparisons. Regression analysis with analysis of covariance (ANCOVA) was used to evaluate the relationship between GAL-3 and influential variables. Correlations of GAL-3 with comorbidities and biomarkers were assessed by Spearman rank correlation, with partial correlations used for controlling of potential confounding variables. Backward linear regression was used for the selection of candidate variables, as it permits all the potential predictors to be considered and it allows for the possibility of a set of variables being evaluated for predictive capability together, even if the individual variables might not be the most predictive. A 2-sided $P < 0.05$ was accepted as statistically significant. Analyses were performed with SAS 9.2 (SAS

Institute Inc, Cary, NC) and graphics generated with SigmaPlot 10.0 (San Jose, CA).

Results

Baseline Characteristics

The mean age of all participants was 62 ± 13 years; 61% were male, and 53% were black. The 6 groups did not differ with regard to race, smoking status, hypertension, ischemic heart disease, cerebrovascular disease, diastolic blood pressure, heart rate, or body mass index. As expected, age and systolic blood pressure were higher and female sex was more common in patients with HFpEF than in those with HFrEF. In the CKD group, the predominant origins of renal insufficiency were hypertension (42%) and diabetes (25%) (Table 1).

Table 1. Baseline Characteristics (N=119)

	Controls (n=32)	HFrEF		HFpEF		CKD (n=12)
		Stable (n=20)	Decompensated (n=21)	Stable (n=18)	Decompensated(n=16)	
Age,* y	59±11	60±12	61±13	69±10	68±15	58±15
Female,† n (%)	15 (47)	1 (5)	4 (19)	10 (56)	10 (63)	7 (58)
Black race, n (%)	9 (28)	10 (50)	15 (71)	11 (61)	11 (69)	7 (58)
BMI, kg/m ²	31±5	31±8	31±7	33±8	34±7	29±6
SBP,* mm Hg	126±14	120±26	130±23	136±20	138±19	131±14
DBP, mm Hg	74±7	71±9	77±11	71±10	73±13	74±11
HR,* bpm	74±7	67±11	77±14	68±13	73±12	73±10
LVEF,* %	62±4	25±11	26±12	62±7	61±7	62±6
NYHA class†						
I	—	4 (20)	0 (0)	10 (56)	0 (0)	—
II	—	14 (70)	0 (0)	8 (44)	0 (0)	—
III	—	2 (10)	5 (24)	0	9 (54)	—
IV	—	0 (0)	16 (76)	0	7 (46)	—
Smoker, n (%)	4 (13)	4 (20)	8 (38)	4 (22)	3 (19)	2 (18)
Hypertension, n (%)	22 (69)	16 (80)	17 (81)	16 (90)	15 (94)	8 (67)
Diabetes mellitus, n (%)	10 (31)	13 (65)	11 (52)	10 (56)	12 (75)	6 (50)
Cerebrovascular disease, n (%)	2 (6)	3 (15)	1 (5)	4 (22)	4 (25)	0 (0)
Ischemic heart disease, n (%)	10 (32)	10 (50)	10 (48)	5 (28)	4 (25)	2 (17)
eGFR,* mL/min per 1.73 m ²	86±25	69±30	68±32	46±23	54±30	33±16
Creatinine,* mg/dL	0.9±0.3	1.7±2.0	1.5±0.8	1.8±0.9	1.6±1.0	2.7 ± 2.2
Plasma NT-proBNP,* fmol/mL	536±382	879±769	3496±2361	1262±602	2269±1337	N/A
Plasma GAL-3,* ng/mL	14±4	21±11	23±12	23±9	22±10	36±11

HFrEF indicates HF with reduced LVEF; HFpEF, HF with preserved LVEF; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVEF, left ventricular ejection fraction; NYHA Class, New York Heart Association Functional Class; and eGFR, estimated glomerular filtration rate.

* $P < 0.001$ for continuous variables compared across groups by the Kruskal-Wallis test.

† $P < 0.001$ for categorical variables compared across groups by χ^2 test or Fisher exact test.

The mean of the eGFRs in the 4 HF groups (60 ± 30 mL/min per 1.73 m²) was lower than in the non-HF control group (86 ± 25 mL/min per 1.73 m², $P < 0.001$), as was the eGFR in the CKD group (34 ± 16 mL/min per 1.73 m², $P < 0.001$). Plasma NT-proBNP, which was 536 ± 382 fmol/mL in control patients without HF, was moderately elevated in patients with stable HFrEF and HFpEF and markedly elevated in patients with decompensated HFrEF and HFpEF.

GAL-3 in HF Groups

Plasma GAL-3 was 14 ± 4 ng/mL in control patients without HF and was elevated in patients with stable (21 ± 11 ng/mL) and decompensated (23 ± 12 ng/mL) HFrEF and in patients with stable (23 ± 9 ng/mL) and decompensated (22 ± 10 ng/mL) HFpEF ($P < 0.05$ versus control patients without HF for all). There were no differences among any of the HF groups (Figure 1). Across all HF groups, plasma GAL-3 did not correlate with age, sex, diabetes, history of coronary artery disease, hypertension, smoking history, systolic blood pressure, diastolic blood pressure, or body mass index. However, in patients with HF, GAL-3 correlated with NT-proBNP ($r = 0.44$, $P < 0.001$). The relationship between GAL-3 and NT-pro-BNP persisted but was substantially weakened after adjustment for age and eGFR ($r = 0.23$, $P = 0.06$).

In the combined HF groups, GAL-3 was inversely correlated with eGFR ($r = -0.75$, $P < 0.001$) (Figure 2A). This relationship remained strong after adjustment for age, LVEF, and NT-proBNP ($r = -0.64$, $P < 0.001$).

Plasma GAL-3 Versus Type of HF and Compensation State

GAL-3 was elevated in patients with both HFpEF and HFrEF compared to control patients without HF but did not differ between HFpEF and HFrEF ($P = 0.37$) (Figure 1A). Likewise, plasma GAL-3 did not differ between patients with decompensated versus stable HF ($P = 0.75$) (Figure 1B).

Relationship to Renal Function

Mean GAL-3 in the CKD group (36 ± 11 ng/mL) was higher than in the non-HF control group (14 ± 4 ng/mL) or the HF groups (combined mean = 22 ± 10 ng/mL). When data from all 6 groups (non-HF controls, HF, CKD) were pooled, a striking inverse curvilinear relationship was apparent for GAL-3 versus eGFR ($r = -0.77$, $P < 0.001$). This relationship was significant both in the patients with HF ($r = -0.75$, $P < 0.001$) (Figure 2A) and in the patients without HF (ie, the combined controls without HF and the patients without HF but with impaired renal function) ($r = -0.82$, $P < 0.001$) (Figure 2B). The relationships between GAL-3 and eGFR were identical in the

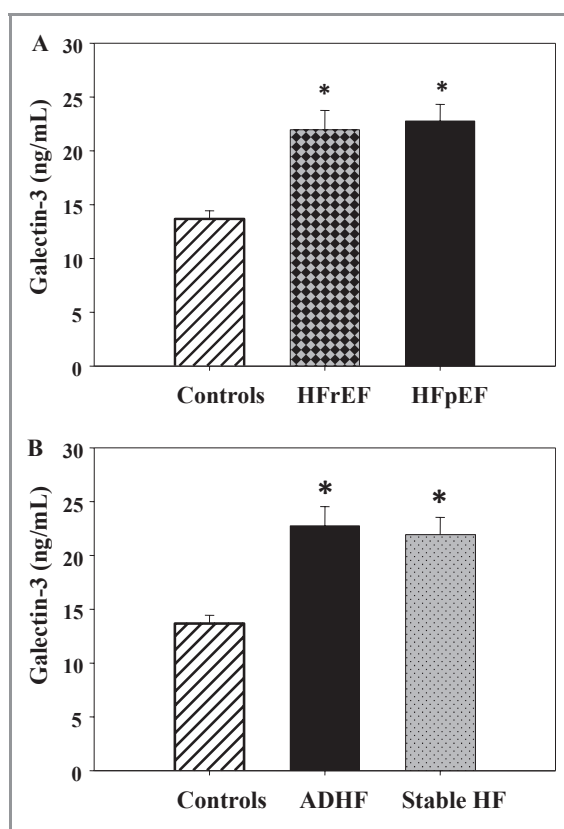


Figure 1. Relationship of plasma GAL-3 levels to the type of HF (A) or level of decompensation (B). A, Plasma GAL-3 in 32 controls without HF, 40 patients with HF with reduced LVEF (HFrEF), and 35 patients with HF with preserved LVEF (HFpEF). GAL-3 was elevated to a similar degree in patients with HFrEF vs HFpEF. * $P < 0.05$ vs control. B, Plasma GAL-3 in 32 controls without HF, 37 patients admitted to the hospital with acute decompensated HF (ADHF), and 38 ambulatory patients with chronic stable HF. GAL-3 was elevated to a similar degree in patients with decompensated vs stable HF. * $P < 0.05$ vs control.

HF and non-HF groups (Figure 2C). By linear regression analysis of the relationship between eGFR and GAL-3, the β -coefficient of eGFR is -0.025 (95% confidence interval, -0.022 to -0.023 , $P < 0.001$, per standard deviation of log GAL-3) with $R^2 = 0.60$. When HF status is added to the regression, the β -coefficient of eGFR is unchanged (-0.025 ; $R^2 = 0.60$). Backward linear regression of log GAL-3 in HF patients was performed with candidate predictors of age, LVEF, sex, and eGFR. With a significance level of 0.05 for variable retention in the model, only eGFR remained predictive of GAL-3 (eGFR: β -coefficient -0.022 ; 95% CI, -0.020 to -0.024 ; $P < 0.001$, per standard deviation of log GAL-3).

Discussion

The major findings of this study are that (1) the inverse relationship between plasma GAL-3 and renal function is

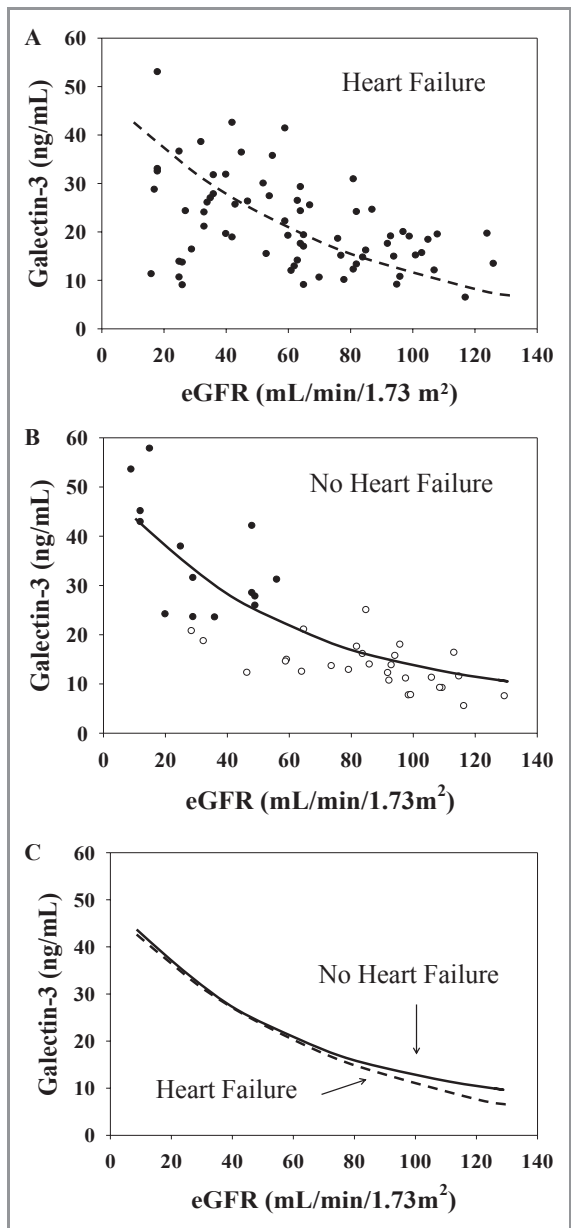


Figure 2. Relationship between plasma GAL-3 and renal function in patients with HF (A) and patients without HF (B). A, Strong curvilinear relationship ($r=-0.75$, $P<0.001$) between plasma GAL-3 and estimated glomerular filtration rate (eGFR) in 75 patients with HF depicted in Figure 1. B, Strong curvilinear relationship ($r=-0.82$, $P<0.001$) between GAL-3 and eGFR in 44 subjects without HF over a range of renal functions comparable to the HF groups. Open circles indicate control subjects; closed circles, patients with CKD. C, Similarity of the relationship between GAL-3 and eGFR in subjects with HF (dashed line) or without HF (solid line) over comparable ranges of renal functions.

independent of the level of clinical decompensation or the pathophysiology of HF, and (2) the presence or absence of clinical HF has little or no effect on the relationship between GAL-3 and renal function.

Relationship to Clinical Compensation

Brain natriuretic peptides, including NT-proBNP, are the most frequently used clinical biomarkers in patients with HF, in whom the elevation in plasma brain natriuretic peptides is strongly related to the severity of clinical decompensation.^{24,25} This relationship is well illustrated in the present study, in which patients were prospectively categorized according to clinical status. In patients with chronic stable HF, NT-proBNP was only modestly (2- to 3-fold) elevated compared to control patients without HF, whereas in decompensated patients, NT-proBNP was markedly (5- to 7-fold) elevated, which thus confirms the prospective clinical categorization used in this study.

Prior studies have not examined the relationship between plasma GAL-3 and the severity of decompensation in HFrEF and HFpEF in a prospective manner. Recently, we observed that episodes of decompensation are associated with increased plasma levels of 3 biomarkers related to the interstitial matrix, possibly reflecting an increase in matrix turnover.²⁶ Because GAL-3 is associated with interstitial fibrosis,^{7,8} it seemed likely that plasma GAL-3 might also increase transiently with an episode of decompensation. Contrary to our thesis, GAL-3 was not affected by episodes of decompensation, regardless of the type of HF (HFrEF or HFpEF).

Relationship to HF Pathophysiology

Prior studies of plasma GAL-3 in HF have focused primarily on patients with HFrEF^{13,18} or on mixed populations of patients with HFrEF and HFpEF.^{12,14,19} No prior study has systematically compared plasma GAL-3 levels between HFpEF and HFrEF in patients with both stable and decompensated HF. Animal studies suggest that GAL-3 is involved in the fibrotic process, and limited clinical data support the notion that plasma GAL-3 is reflective of myocardial fibrosis.²⁷ Because HFpEF is associated with interstitial fibrosis²⁸ and increased plasma levels of biomarkers of matrix turnover,^{29,30} it seemed likely that GAL-3 would be more elevated in patients with HFpEF than in those with HFrEF. However, GAL-3 levels were not different in patients with HFrEF versus HFpEF. As discussed previously, GAL-3 was not affected by the level of compensation, and likewise, GAL-3 levels in patients with HFrEF and HFpEF were similar regardless of the level of compensation. Thus, although plasma GAL-3 is elevated in patients with HF, the degree of elevation is not related to the severity of decompensation or the underlying type of HF.

Relationship to Renal Function

Plasma GAL-3 correlated strongly ($r=-0.75$, $P<0.001$) with eGFR across all of the HF groups, regardless of type or severity of HF (see Figure 2A). Furthermore, this relationship

remained strong after adjustment for age, LVEF, and NT-proBNP. In further support of the importance of renal function in predicting GAL-3, eGFR was the strongest predictor of GAL-3 levels in patients with HF when evaluated with other predictors, such as age, LVEF, and sex in backwards linear regression.

To further characterize the relationship of GAL-3 with renal function in the absence of HF, we measured GAL-3 in a group of patients with moderate renal insufficiency due to CKD but no HF. As with the patients who had HF, GAL-3 correlated strongly ($r=-0.82$, $P<0.001$) with eGFR in patients without HF across a wide range of eGFRs that was comparable to the range in the HF groups. Our data thus indicate that renal function is a major determinant of GAL-3 levels, both in patients with HF and in patients without HF. Of note, the curvilinear relationships between GAL-3 and eGFR were superimposable for patients with and without HF (see Figure 2C), which suggests that renal impairment, rather than HF, could be the major determinant of GAL-3 in patients with HF.

There are several potential mechanisms for increased GAL-3 levels in the setting of renal impairment. First, it is possible that GAL-3 (molecular weight ≈ 30 kDa) clearance is via the kidney and thus serves primarily as a marker of reduced renal function. Second, there could be increased production of GAL-3 by the kidney.^{31,32} Third, it is possible that GAL-3 is produced in the kidney and exerts profibrotic effects resulting in renal function impairment. However, evidence also exists that GAL-3 could protect the kidney from ischemia/reperfusion^{32,33} and in CKD.³⁴ Finally, it is possible that increased GAL-3 in HF reflects production by organs other than the heart and kidneys, particularly in the setting of systemic inflammation^{35–37} that can occur in both HF and CKD.³⁸

Limitations

Certain limitations of this study should be considered. First, the relatively small number of patients might have reduced the ability to distinguish differences between patient groups with stable versus decompensated HF or HF_{rEF} versus HF_{pEF}. A second limitation is the difficulty of controlling for all factors that might affect GAL-3 level. Although it is possible that these limitations might have led to underestimation of the differences between groups, we think it is unlikely that important differences were missed, as the data were sufficiently robust to detect the striking effect of renal function on plasma GAL-3 in patients with or without HF. Finally, because of the sample sizes, it was not possible to perform a formal interaction test for the effect of HF on the relationship of GAL-3 to eGFR. Nevertheless, as Figure 2C illustrates, the influence of HF, if any, seems to be small.

Summary

GAL-3 recently was introduced as a biomarker for the assessment of prognosis in patients with HF.¹ Here we demonstrate that renal impairment is a major determinant of plasma GAL-3 in patients with or without HF. Concentrations of plasma GAL-3 do not seem to depend on the level of compensation or type of HF. Furthermore, the relationship between GAL-3 and renal function seems to be affected little or not at all by the presence or absence of clinical HF. These observations raise important considerations with regard to both the use of GAL-3 in clinical practice and the interpretation of GAL-3 values in outcome trials in patients with HF.

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Disclosures

The funding sources were not involved in the study design, conduct, analysis, interpretation of the data, preparation of the manuscript, or decision to submit the manuscript for publication.

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