The Prognostic Value of Plasma Galectin 3 in HFrEF Depends on the Etiology of Heart Failure: a Cohort Study and Animal Experiment

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Research
Abstract

**Background:** Although plasma galectin 3 (Gal-3) has been investigated in many previous studies, its prognostic value has not yet been determined. However, many studies had found that plasma and cardiac levels of Gal-3 are different within different animal models of heart failure (HF).

**Aim:** The aim of the present study was to evaluate the prognostic value of plasma Gal-3 for HF originating from different causes.

**Methods:** We examined the plasma levels and expression of Gal-3 in cardiac tissues in two transgenic (TG) strains of mice with cardiomyocyte-restricted overexpression of either β2-adrenergic receptor (β2-AR TG) or Mammalian sterile 20-like kinase 1 (Mst1-TG) in the present study. Age-matched non-transgenic (nTG) littermates were used as controls. Additionally, 166 patients suffering from heart failure with reduced ejection fraction (HFrEF) in two hospitals within the Shaanxi province were included in the present study. These patients were treated in accordance with the Chinese HF guidelines of 2014. Subsequently, these patients were followed up for 50 months, during which we analyzed the prediction value of baseline Gal-3 to endpoints in these patients via Cox and Kapla-Meir analyses.

**Results:** Gal-3 was localized in the cytoplasm and nucleus of cardiomyocytes, often forming aggregates in Mst1TG mice. Extracellular Gal-3 staining was uncommon in Mst1-TG hearts. However, in β2-AR TG mice, although Gal-3 was also expressed in myocardial cells, it is more highly expressed in interstitial cells (e.g., fibroblasts and macrophages). Plasma Gal-3 was comparable between nTG and Mst1 TG mice, however, plasma Gal-3 was higher in β2-AR TG mice than nTG mice. In HFrEF patients cohort, the median Gal-3 concentration was 158.42 pg/mL. All participants were divided into two groups according to their Gal-3 levels. There were no statistical differences between the two groups in terms of gender, hypertension history, diabetes history, treatments, death, re-hospitalization and composite endpoint events. However, patients with Gal-3 plasma concentrations above the median were older (P=0.043), had lower plasma hemoglobin (P = 0.002), but higher plasma creatinine (P=0.011), tissue inhibitor of metalloproteinases 1 (TIMP-1) (P=0.002), left ventricular end systolic diameter (L VESD) (P = 0.036), left ventricular end-systolic volumes (LVESV) (P=0.043) and end-diastolic, and left ventricular end-diastolic volumes (LVEDV) (P=0.036). Spearmen correlation analysis revealed that Gal-3 was positively correlated with TIMP-1 (r=0.396, P<0.001), LVESV (r=0.181, P=0.020) and LVEDV (r=0.190, P=0.015). During a 50-month follow-up, 43 deaths, 97 unplanned re-hospitalizations, and 111 composite endpoint events occurred. Cox analysis demonstrated that although Gal-3 did not provide any prognostic value in either total-HF subjects or coronary-heart-disease (CHD) patients, it did provide prognostic value in non-CHD patients.

**Conclusion:** Although plasma concentrations of Gal-3 were associated with TIMP-1 and echocardiographic parameters, the prognostic value of plasma Gal-3 in HFrEF depended on the etiology of HF.
Highlight

The prognostic value of Galectin-3 in HFrEF

Introduction

Heart failure (HF) is a disease responsible for high morbidity and mortality regardless of therapies\(^1,2\). Hence, there is an increasing need for early diagnosis, better prognostic evaluation and management of HF. Thus, as indicators of pathological processes and responses to therapeutic interventions, circulating blood biomarkers have been increasingly studied due to their noninvasive determinations that tend to be sufficiently sensitive and accurate. Although there many different available biomarkers (e.g., NT-proBNP\(^3\), GDF-15\(^4\)), there are also multiple factors that affect the prognostic values of these biomarkers.

Galectin 3 (Gal-3) is a soluble \(\beta\)-galactoside-binding protein. It is expressed in epithelial and inflammatory cells in several organs and is located both intracellularly and extracellularly\(^5,6\). Gal-3 is involved in cellular functions related to cell adhesion\(^7,8\), proliferation\(^9\), and differentiation\(^10\text{--}12\), and is considered a biomarker of cardiac fibrosis and remodeling\(^5,13,14\). In the myocardium, Gal-3 is primarily expressed in fibroblasts, and macrophages that play an important role in the formation of myocardial fibrosis through activation of fibroblasts\(^15\) have been linked to fibrosis in a spectrum of medical conditions, including HF. Although many previous studies have demonstrated elevated plasma concentrations of Gal-3 in both acute and chronic HF, the prognostic value of Gal-3 in predicting re-hospitalization and mortality has not yet been determined. We et al\(^16\) and Du et al\(^17\) found that plasma and cardiac levels of Gal-3 were different across distinct HFs caused by different etiologies in experimental animals. Therefore, we hypothesized that the prognostic value of plasma Gal-3 depends on the etiology of HF. Hence, our present study evaluated the prognostic value of plasma Gal-3 across distinct HFs with different causes.

Methods

This study was completed in mouse models and in human HF patients.

Animals

Two transgenic (TG) strains of mice with cardiomyocyte-restricted overexpression of either \(\beta_2\)-adrenergic receptor (\(\beta_2\)-AR TG) or Mammalian sterile 20-like kinase 1 (Mst1 TG) were used in the present study. Our previous works have characterized cardiomyopathic phenotypes of both models\(^16,18\text{--}20\). All strains of mice were from the same C57Bl/6 genetic background. Only male mice were studied. Age-matched non-transgenic (nTG) littermates were used as controls. Mice were housed in standard conditions with food and water provided \textit{ad libitum}. All experimental procedures were approved by a local animal ethics committee in compliance with both the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition) and the ARRIVE guidelines.
Subjects

This protocol was approved by the ethics committee of the First Affiliated Hospital of Xi’an Jiaotong University (Shaanxi 710061, China) and was in accordance with the Helsinki Declaration’s guidelines. Informed consent was obtained for all participants. The cohort study consisted of chronic HF patients, aged between 18–80 years, who were diagnosed with heart failure with reduced ejection fraction (HFrEF) in the Department of Cardiovascular Medicine at Xunyi Hospital and Jingyang Hospital from May 2014 to May 2015. Patients were then followed up for a period of 50 months and were evaluated for the development of major adverse cardiovascular events (MACEs). Patients were excluded from the present study if they had acute HF, active neoplasia, acute myocardial infarction, acute or chronic liver disease (alanine aminotransferase level >5 times the upper normal limit), acute stroke, serious kidney disease, chronic consumption disease, thyroid dysfunction, fibrotic pathologies (e.g., pulmonary fibrosis, collagenases), and/or cancer. Following these exclusion criteria, a total of 166 HF patients were recruited. All participants were divided into two groups (group 1 and group 2) according to their Gal-3 levels. Then, based on their clinical features, patients from each group were further divided into two subgroups, with coronary-heart-disease (CHD) group and without CHD group.

Histological and plasma analyses in mice

Blood was collected in heparin-containing vials when mice were killed, centrifuged at 4°C (3,000 rpm in 20 min), and stored at −80°C. Plasma Gal-3 levels were detected by a mice Galectin-3 Quantikine ELISA Kit (R&D Systems Inc., Minneapolis, MN, USA) in twin duplicates wells, following protocols provided by the manufacturer. And then Paraffin-embedded LV sections (6 μm) were prepared and used for Gal-3 immunofluorescent staining. For Gal-3 immunofluorescent staining, after samples had been dewaxed, heat-induced antigen retrieval and permeabilization were carried out (with 10 mM of Na-citrate buffer containing 0.05% Tween 20; pH 6.0; 95°C for 25 min) followed by blocking with DAKO Protein Block (X0909, Agilent, 1 h at room temperature). Sections were incubated with primary goat anti-mouse Gal-3 (1:100, AF1197, R&D Systems) overnight at 4°C, after which they were incubated with the secondary antibody, Alexa Fluor 594 donkey anti-goat IgG (1:200, A11058, Invitrogen by Thermo Fisher Scientific). The cardiomyocyte boundary was revealed by wheat-germ-agglutinin FITC staining (1:80, FL-1021, Vector Labs, 1 h at room temperature). Images were acquired with an Olympus BX61 fluorescent microscope.

Clinical measurements

Investigators and a trained interviewer collected all of the clinical data. The trained interviewer collected patient information, including demographic data, past medical history, history of cardiovascular diseases, the Minnesota Living with Heart Failure Questionnaire (MLHFQ), New York Heart Association (NYHA) functional class, smoking behavior, and alcohol abuse. Smoking was defined as smoking cigarettes within one month of the indexed hospital admission. Hypertension was defined as a cuff blood pressure ≥140/90 mmHg and/or the current use of antihypertensive medications. Subjects were also questioned about their past histories of diabetes mellitus and their current use of anti-diabetic drugs. Diagnosis of diabetes was confirmed if plasma fasting glucose was ≥7.0 mM (or if the 2-h postprandial glucose was >
11.1 mM) or if there was current use of anti-diabetic medication. Anthropometric measurements, such as body weight (kg) and height (m), were taken during the first visit. Body mass index (BMI) was calculated as weight divided by height squared.

**Analysis of patients blood parameters**

Blood was collected from each patient at admission. After overnight fasting, between 6–7 a.m., blood from the median cubital vein was drawn into ethylenediaminetetraacetic acid (EDTA)-containing tubes. Plasma was separated within 2 h after collection. Blood parameters were measured at the Central Clinical Laboratory of the First Affiliated Hospital of Xi’an Jiaotong University, including hemoglobin (HB), creatinine (CR), urea nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Plasma samples for later analyses (i.e., to detect Gal-3 levels) were collected in EDTA-containing vials, centrifuged at 4°C (3,000 rpm in 10 min), and stored at −80°C.

Plasma Gal-3 levels were detected by a Human Galectin-3 Quantikine ELISA Kit (R&D Systems Inc., Minneapolis, MN, USA) in duplicates wells, following protocols provided by the manufacturer; the mean serum Gal-3 value was calculated as the final level. The detection range of the plasma Gal-3 immunoassay was 0–4,000 pg/mL. Plasma TIMP-1 levels were detected by a human TIMP-1 Quantikine ELISA Kit (Abbkine, Inc., China) in duplicates wells, following protocols provided by the manufacturer, the mean serum TIMP-1 value was calculated as the final level. The calibration range of serum TIMP-1 was 31.25–2,000 ng/mL, and the limit of detection was 16 ng/mL.

**Echocardiographs and electrocardiograms**

Echocardiographs were performed with a Phillips iE33 system by a single trained operator blinded to the Gal-3 plasma concentration of each subject. All echo data were analyzed by a single operator to limit inter-observer variability. The left ventricular ejection fraction (LVEF) was calculated by the Simpson biplane model. The following standard parameters were collected: left ventricular end-systolic and end-diastolic volumes (LVESV and LVEDV); left ventricular end-systolic and end-diastolic dimensions (LVESD and LVEDD); and left ventricular fraction shortness (LVFS). The 12 electrocardiographic leads were made up of three standard limb leads (I, II, and III), augmented limb leads (aVR, aVL and aVF), and six precordial leads (V1, V2, V3, V4, V5, and V6). The QT interval was best measured between the beginning of the Q wave until the end of the T wave in lead II.

**Treatments and evaluations of patient outcomes**

All HF patients were actively followed up at average times of 1, 3, 6, and 50 months after the initiation of treatments. Follow-up information was completed for all 166 patients (100%). Information was obtained by face-to-face interviews or telephone conversations. Information regarding secondary cardiovascular events and treatments since the start of the treatment in the present study was obtained. Cardiovascular events were defined as either MACEs as the main cause of death, re-hospitalization because of HF, or composite endpoint events. All patients received β-blockers, as well as angiotensin-converting enzyme
inhibitors (ACEI) or angiotensin receptor blockers (ARB), according to the China HF guidelines of 2014, unless there were contraindications to these drugs. Mineralocorticoid-receptor antagonists, diuretics, and digoxin were prescribed to patients who had corresponding indications according to the China HF guidelines of 2014.

**Statistical analysis**

Analyses were performed using SPSS version 13.0. Normally distributed values are presented as mean±standard deviations (SDs), and differences between groups were determined using Student’s *t* tests. Variables with a skewed normal distribution are presented as medians (inter-quartile range), and between-group differences for these variables were determined using Rank-Sum tests. Categorical variables are presented as percentages, and differences between groups were tested using Chi-squared tests. MACE-rate estimates were generated via the Kaplan-Meier method. Cox proportional hazards modeling was used to assess the relative importance of baseline risk factors to the resulting endpoints. Hazard ratios (HR) are presented, with 95% CIs, to show the risk of an event when a given factor was present. Significance was defined at the 5% level using a two-tailed statistical test.

**Results**

**Myocardial and serum Gal-3 expression levels in cardiomyopathic mice**

Our previous studies showed that myocardial Gal-3 concentrations were higher in both Mst1-TG mice and β2-AR-TG mice compared to that in nTG mice.\(^{[16,19,21,22]}\) In keeping with our previous findings, whereas the plasma Gal-3 concentration in β2-AR-TG mice was significantly elevated versus nTG mice, Mst1-TG mice showed no change in plasma Gal-3 concentration compared with that of respective nTG group (Fig. 1). By immunohistochemistry, we found that Gal-3 was localized in the cytoplasm and nucleus of cardiomyocytes, and often formed aggregates in Mst1 TG mice. Extracellular Gal-3 staining was uncommon in Mst1-Tg hearts. However, in β2-AR TG mice, although certain number of cardiomyocytes were positively stained by Gal-3, Gal-3 was more often expressed in interstitial cells (e.g., fibroblasts and macrophages) (Fig. 2).

**Baseline characteristics of HF patients**

Our study cohort included 105 (48.9%) men and 61 (51.1%) women. Plasma Gal-3 concentrations were between 23.88–1157.63 pg/mL, and the median Gal-3 concentration was 158.42 pg/mL. All participants were divided into two groups according to their Gal-3 levels. Next, the clinical data were compared between these two groups. As shown in Table 1, there were no statistical differences between the two groups in terms of gender, hypertension history, DM history, treatments, or MACEs. However, patients with Gal-3 plasma concentrations above the median were older (*P* = 0.043). Table 1 also demonstrates that patients with increased Gal-3 plasma concentrations had lower plasma HB (*P* = 0.002) but higher plasma CR (*P* = 0.011), TIMP-1 (*P* = 0.002), LVESD (*P* = 0.036), LVESV (*P* = 0.043), and LVEDV (*P* = 0.036).
Table 1  
Baseline characteristics of all HF patients.

|                          | Below median Gal-3 (n = 83) | Above median Gal-3 (n = 83) | F/X | P     |
|--------------------------|-----------------------------|-----------------------------|-----|-------|
| Age (years)              | 60.6 ± 9.155                | 63.61 ± 9.335               | 2.043 | 0.043 |
| Gender (M/F)             | 55/28                       | 50/33                       | 0.648 | 0.421 |
| Hypertension (%)         | 29.3                        | 39.0                        | 1.735 | 0.188 |
| Diabetes Mellitus (%)    | 7.2                         | 7.2                         | 0.004 | 0.948 |
| Smoking (%)              | 57.8                        | 47.0                        | 1.956 | 0.162 |
| Alcohol consumption (%)  | 41.5                        | 41.0                        | 0.004 | 0.948 |
| Coronary heart disease (%) | 34.9                   | 48.2                        | 3.001 | 0.083 |
| HF history (years)       | 4.0 (2.0, 6.0)              | 4.0 (3.0, 7.0)              | 0.054 |       |
| MLHFQ                    | 25.0 (14.0, 34.0)           | 29.0 (16.0, 38)             | 0.182 |       |
| SBP (mmHg)               | 122 ± 19                    | 122 ± 23                    | 0.1   | 0.920 |
| DBP (mmHg)               | 77 ± 12                     | 78 ± 11                     | 0.247 | 0.805 |
| CR (umol/L)              | 78.0 ± 12.1                 | 84.3 ± 18.5                 | 2.582 | 0.011 |
| BUN (mmol/L)             | 6.64 ± 1.49                 | 7.14 ± 2.18                 | 1.642 | 0.103 |
| HB (g/L)                 | 151.3 ± 21.7                | 141.9 ± 15.7                | 3.193 | 0.002 |
| AST (U/L)                | 24.6 ± 8.3                  | 24.1 ± 9.4                  | 0.294 | 0.108 |
| ALT (U/L)                | 21.5 ± 10.6                 | 20.9 ± 11.6                 | 0.261 | 0.795 |
| TIMP-1 (ng/mL)           | 113.3 ± 89.7                | 160.1 ± 103.7               | 3.112 | 0.002 |
| QT interval (ms)         | 419 ± 49                    | 422 ± 50                    | 0.237 | 0.813 |
| BMI (Kg/m²)              | 22.88 ± 3.31                | 23.35 ± 3.78                | 0.834 | 0.406 |
| Heart Rate (bpm)         | 78.5 ± 14.9                 | 78.4 ± 18.3                 | 0.042 | 0.967 |
| LVEF (%)                 | 35.3 ± 7.1                  | 35.5 ± 7.7                  | 0.141 | 0.888 |

Data are mean (SD) or n (%) unless otherwise stated. F: female, M: male, MLHQ: the Minnesota Living with Heart Failure Questionnaire, SBP: systolic blood pressure, DBP: diastolic blood pressure, CR: creatinine, BUN: urea nitrogen, HB: hemoglobin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TIMP-1: tissue inhibitor of metalloproteinases 1, BMI: Body mass index, LVEF: left ventricular ejection fraction, LVESD: left ventricular end-systolic dimension, LVEDD: left ventricular end-diastolic dimension, LVEF: left ventricular end-systolic volumes, LVEDV: left ventricular end-diastolic volumes, FS: left ventricular fractional shortening, NYHA: New York Heart Association ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blockers, ARNI: Sacubitril/Valsartan, MRA: mineralocorticoid receptors antagonist.
### Relationships between plasma Gal-3 levels and clinical characteristics

Figure 3 illustrates the associations between both Gal-3 and echocardiographic variables and the associations between Gal-3 and myocardial fibrosis biomarkers. Following spearmen correlation

|                      | Below median Gal-3 (n = 83) | Above median Gal-3 (n = 83) | F/X | P    |
|----------------------|-----------------------------|----------------------------|-----|------|
| LVESD (mm)           | 56.43 ± 8.57                | 57.29 ± 10.02              | 2.116 | 0.036 |
| LVEDD (mm)           | 68.92 ± 8.38                | 70.19 ± 9.43               | 0.922 | 0.358 |
| LVESV (ml)           | 135.37 ± 55.17              | 155.50 ± 70.48             | 2.037 | 0.043 |
| LVEDV (ml)           | 199.04 ± 73.09              | 225.21 ± 84.85             | 2.116 | 0.036 |
| Fractional Shortening (%) | 17.88 ± 4.10                 | 17.80 ± 4.82               | 0.116 | 0.908 |
| NYHA functional class |                            |                            |      |      |
| I                    | 20.5                        | 10.8                       | 3.264 | 0.353 |
| II                   | 53.0                        | 60.2                       |      |      |
| III                  | 22.9                        | 26.5                       |      |      |
| IV                   | 3.6                         | 2.4                        |      |      |
| β-blocker (%)        | 79.5                        | 81.9                       | 0.155 | 0.694 |
| ACEI/ARB/ARNI (%)    | 90.4                        | 89.2                       | 0.066 | 0.798 |
| MRA (%)              | 73.5                        | 79.5                       | 0.838 | 0.360 |
| Digoxin (%)          | 19.3                        | 27.7                       | 1.642 | 0.2   |
| Diuretics (%)        | 55.4                        | 59.0                       | 0.221 | 0.638 |
| Death rate (%)       | 20.3                        | 31.3                       | 2.542 | 0.111 |
| Re-hospitalization rate (%) | 52.4                        | 65.4                       | 2.842 | 0.092 |
| Composite-endpoint event | 61.4                        | 72.3                       | 2.202 | 0.138 |

Data are mean (SD) or n (%) unless otherwise stated. F: female, M: male, MLHFQ: the Minnesota Living with Heart Failure Questionnaire, SBP: systolic blood pressure, DBP: diastolic blood pressure, CR: creatinine, BUN: urea nitrogen, HB: hemoglobin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TIMP-1: tissue inhibitor of metalloproteinases 1, BMI:Body mass index, LVEF: left ventricular ejection fraction, LVESD: left ventricular end-systolic dimension, LVEDD: left ventricular end-diastolic dimension, LVESV left ventricular end-systolic volumes, LVEDV: left ventricular end-diastolic volume, FS: left ventricular fractional shortening, NYHA: New York Heart Association ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blockers, ARNI: Sacubitril/ Valsartan, MRA: mineralocorticoid receptors antagonist.
analysis, Gal-3 was positively correlated with TIMP-1 \((r = 0.396, P < 0.001)\), LVESV \((r = 0.181, P = 0.020)\), and LVEDV \((r = 0.190, P = 0.015)\).

**Prognostic value of plasma Gal-3 levels**

During a 50-month follow-up, 43 deaths, 97 unplanned re-hospitalizations, and 111 composite endpoint events including death and unplanned re-hospitalizations occurred. Following univariate Cox analysis, Gal-3 did not provide any prognostic value when all HF subjects were analyzed together (Fig. 4 and Table 2). Furthermore, we performed stratified analysis in accordance with or without CHD subgroups. COX regression analysis and Kaplan-Meier analysis were performed. We found that Gal-3 did not provide any prognostic value in CHD participants. In contrast, as shown in Fig. 5 and Table 3, Gal-3 did predict prognoses without CHD subjects.

| Table 2 |
|---------|
| Predictive value of baseline plasma Gal-3 to long-term outcomes in all HFrEF patients. |
| HR (95% CI) | P |
|-----------------------------|-----|
| Death | 1.769 (0.957, 3.268) | 0.069 |
| Re-hospitalization | 1.454 (0.968, 2.184) | 0.071 |
| Composite-endpoint event | 1.433 (0.983, 2.088) | 0.061 |
| HR: hazard ratio, CI: confidence intervals. |

| Table 3 |
|---------|
| Predictive value of baseline plasma Gal-3 to long-term outcomes in HFrEF with or without CHD patients. |
| HFrEF without CHD patients | HFrEF with CHD patients |
|---------------------------|------------------------|
| HR (95% CI) | P | HR (95% CI) | P |
| Death | 2.292 (1.071, 4.905) | 0.033 | 1.899 (0.664, 5.435) | 0.232 |
| Re-hospitalization | 1.756 (1.021, 3.018) | 0.042 | 1.473 (0.799, 2.716) | 0.215 |
| Composite-endpoint event | 1.673 (1.022, 2.740) | 0.041 | 1.545 (0.858, 2.780) | 0.147 |
| HFrEF: heart failure with reduced ejection fraction, CHD: coronary heart disease, HR: hazard ratio, CI: confidence intervals. |

**Discussion**

Our present study revealed three primary findings. First, Gal-3 levels in myocardial tissue and plasma were different between two mouse models of cardiomyopathy (i.e., Mst1 TG mice and β2-AR TG mice). Second, plasma concentrations of Gal-3 were associated with TIMP-1 and echocardiographic
parameters. Finally, although plasma concentrations of Gal-3 did not predict prognoses in all participants, it was predictive of prognoses in HFrEF without CHD subjects.

Gal-3 is primarily expressed in fibroblasts and macrophages and is involved in myocardial fibrosis through activation of fibroblasts\(^6,23\). In the present study, we found that Gal-3 was also expressed in cardiomyocytes; moreover, the expression of Gal-3 was different between two mouse models of cardiopathy. In Mst1 TG mice, Gal-3 was primarily expressed in cardiomyocytes, while it was mainly expressed in myocardial interstitial cells in $\beta_2$-AR TG mice. These results suggest that the expression of Gal-3 in myocardial tissue is related to the etiology of HF. The differential expression of Gal-3 in our two mouse models may explain why serum Gal-3 levels in Mst1 TG mice were not significantly increased compared to those in wild-type mice. This phenomenon has also been confirmed in previous studies. Du et al. found that Gal-3 expression was confined to the infarcted area and was localized to both non-cardiomyocytes and cardiomyocytes; importantly, plasma levels of Gal-3 also were transiently elevated at three-days post-infarction, but plasma Gal-3 was not elevated, despite increased cardiac expression and protein levels in TAC mice\(^{17}\).

Myocardial fibrosis is an important pathophysiological mechanism involved in the development and progression of chronic heart failure (CHF)\(^{24-26}\). Collagen synthesis by myocardial fibroblasts is activated in diseases such as CHF and is affected by many determinants (e.g. Gal-3\(^{27,28}\) and TIMP-1\(^{29-31}\)). Zile et al had found that the plasma concentration of TIMP-1 was increased in 1,776 HFrEF patients with NYHA Class-II to -IV symptoms in the PARADIGM-HF trial\(^{32}\). TIMP-1 has been demonstrated to contribute to ventricular remodeling and myocardial apoptosis in experimental HF models\(^{31,33}\). Since Gal-3 has been linked to myocardial fibrosis, it is plausible that elevated plasma concentrations of Gal-3 may also be linked to TIMP-1. Therefore, future studies should further investigate the roles and mechanisms of Gal-3 and TIMP-1 in myocardial fibrosis and HF.

Theoretically, since Gal-3 is involved in myocardial fibrosis, it should be correlated with echocardiographic parameters. In the present study, plasma Gal-3 was positively correlated with LVEDV and LVESV in chronic HFrEF patients. Few prior studies have systematically evaluated the relationship between echocardiographic measures and blood concentrations of Gal-3. The DEAL-HF trial performed serial echocardiographic measures in 240 HF patients with NYHA Class-III and -IV symptoms and found a positive association between increased plasma concentrations of Gal-3 and changes in LVEDV, whereas there was no correlation between baseline LVEDV and Gal-3 levels\(^{34}\). These previous results are different from those of our present study. This discrepancy may be related to the different research subjects in each study. All subjects in the DEAL-HF trial were patients with NYHA Class-III and -IV symptoms, whereas all patients in our present study exhibited NYHA class I–IV symptoms.

Although there have been many studies investigating the relationship between blood levels of Gal-3 and mortality in HF patients\(^{35-37}\), the predictive value of Gal-3 for the prognosis of HF remains to be illusive \(^{38}\). Recently, the PARADIGM-HF trial revealed that baseline and eight-month changes in serum Gal-3
levels did not predict outcomes in HFrEF patients\cite{32}. However, based on the results of animal experiments, we speculate that the predictive value of Gal-3 in the prognosis of HF may be related to the etiology of HF\cite{16–18} and the specific therapies used to treat HF\cite{39}. In the present study, we found that plasma Gal-3 did not predict the mortality in all HF subjects, while it did correlate with mortality in HF without CHD subjects. The expression of Gal-3 in myocardial tissue is affected by inflammation\cite{40,41}, β blockers\cite{39} and the Hippo pathway\cite{22}. Gal-3 expression in myocardial tissue also depends on pathophysiological mechanisms that are independent on the etiologies of HF. This phenomenon may explain different results across studies. However, the strength of the predictive value of Gal-3 in HF requires more animal and clinical studies.

**Limitations**

We examined Gal-3 expression in mice and assessed the predictive value of blood Gal-3 on clinical endpoints in HFrEF patients. However, our present study had several limitations. First, although 166 HFrEF patients were included, there were not enough patients for a sufficient assessment at the 50-month follow-up. Second, we only assessed baseline Gal-3 concentrations in the present study, whereas we did not assess such concentrations after treatments.

**Conclusions**

Although plasma concentrations of Gal-3 were associated with TIMP-1 and echocardiographic parameters, the prognostic value of plasma Gal-3 in HFrEF patients depended on the etiology of HF.

**Abbreviations**

Gal-3: galectin 3, HF: heart failure, HFrEF: heart failure with reduced ejection fraction, ACEI: angiotensin converting enzyme inhibitors, ARB: angiotensin receptor blockers, CHD: coronary heart disease, MLHFQ: the Minnesota Living with Heart Failure Questionnaire, SBP: systolic blood pressure, DBP: diastolic blood pressure, CR: creatinine, BUN: urea nitrogen, HB: hemoglobin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TIMP-1: tissue inhibitor of metalloproteinases 1, BMI: Body mass index, LVEF: left ventricular ejection fraction, LVESD: left ventricular end-systolic dimension, LVEDD: left ventricular end-diastolic dimension, LVESV: left ventricular end-systolic volumes, LVEDV: left ventricular end-diastolic volumes, FS: left ventricular fractional shortening, NYHA: New York Heart Association, ARNI: Sacubitril/Valsartan, MRA: mineralocorticoid receptors antagonist, HR: hazard ratio, CI: confidence intervals, nTG: non-transgenic mice, β2-AR TG: β2-adrenergic receptor transgenic, Mst1-TG: Mammalian sterile 20-like kinase 1 transgenic.

**Declarations**

 Ethics approval and consent to participate
The protocol about patients was approved by the ethics committee of the First Affiliated Hospital of Xi’an Jiaotong University (Shaanxi 710061, China) and was in accordance with the Helsinki Declaration's guidelines. Informed consent was obtained for all participants and families. The mice were introduced from Jackson Laboratory (USA) and informed consent was obtained from this laboratory. These procedures about animal experiments were approved by a local animal ethics committee in compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition) and the ARRIVE guidelines.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Competing interests**

The authors declare no competing interests.

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**Authors’ contributions**

QL, XD, LB, and AM designed the study. QL, SC, YW, TL, YX, JL, QY, and XT followed up with the included patients. QL and SY performed the experiments. QL and RZ collected and analyzed the data. QL prepared the manuscript. All authors read and approved the final manuscript.

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Figures
Figure 1

Plasma concentration of Gal-3 from nTG, Mst1-TG, and β2-AR-TG hearts in mice. Abbreviations are as follows: Gal-3, galectin-3; nTG: non-transgenic mice; b2-AR TG: β2- adrenergic receptor transgenic; Mst1-TG: Mammalian sterile 20-like kinase 1 transgenic.
Immunofluorescent staining of LV sections from wild-type, Mst1-Tg, and β2-AR-Tg hearts in mice. Gal-3 staining: red fluorescence. Merged images also show cell boundaries in green (i.e., wheat-germ agglutinin-FITC staining).

Correlations of serum Gal-3 levels with TIMP-1, LVEDV, and LVESV in HF patients. A) Correlation of Gal-3 with TIMP-1. B) Correlation of Gal-3 with LVESV. C) Correlation of Gal-3 with LVEDV. Abbreviations are as follows: Gal-3, galectin-3; LVESV, left ventricular end systolic volume; LVEDV, left ventricular end diastolic volume.
Figure 4

Kaplan–Meier survival curves according to baseline plasma Gal-3 levels in all HF subjects. A) Death rates according to higher or lower baseline plasma Gal-3 levels. B) Re-hospitalization rates according to higher or lower baseline plasma Gal-3 levels. C) Composite-endpoint event rates according to higher or lower baseline plasma Gal-3 levels. Abbreviations are as follows: Gal-3, Galectin-3.

Figure 5

Kaplan–Meier survival curves according to baseline plasma Gal-3 levels in non-CHD subjects. A) Death rates according to higher or lower baseline plasma Gal-3 levels. B) Re-hospitalization rates according to higher or lower baseline plasma Gal-3 levels. C) Composite-endpoint event rates according to higher or lower baseline plasma Gal-3 levels. Abbreviations are as follows: Gal-3, Galectin-3.