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Circulating Galectin-3 Levels Are Persistently Elevated After Heart Transplantation and Are Associated With Renal Dysfunction

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ABSTRACT

OBJECTIVES This study evaluated changes in serum levels of galectin (Gal)-3 before and after heart transplantation (HTx) and assessed the role of pre-HTx Gal-3 as a biomarker for post-HTx outcomes.

BACKGROUND Gal-3 is a novel biomarker that reflects cardiac remodeling and fibrosis. Elevated serum Gal-3 levels are associated with poor prognosis in heart failure patients. Whether Gal-3 levels change following HTx and the significance of post-HTx outcomes are unknown.

METHODS Serum Gal-3 levels were measured in 62 patients at 118 days (Interquartile Range [IQR]: 23 to 798 days) before and 365 days (IQR: 54 to 767 days) post HTx. Cardiac tissue taken during routine post-HTx endomyocardial biopsy was evaluated to assess the correlation between tissue Gal-3 staining and serum Gal-3 levels and with the presence of myocardial hypertrophy and fibrosis.

RESULTS Serum Gal-3 levels remained significantly elevated (>17.8 ng/ml) in 35 patients (56%) post HTx. There was a significant inverse correlation between Gal-3 levels and glomerular filtration rate measured before and after HTx ($p > 0.005$). There was no association between Gal-3 serum level and Gal-3 staining of myocardial tissue or with the presence of myocyte hypertrophy and interstitial fibrosis post HTx. Elevated pre-HTx Gal-3 levels were associated with reduced post-HTx exercise capacity, but this association was not significant after adjustment for age, body mass index, and glomerular filtration rate.

CONCLUSIONS This is the first study to demonstrate the fact that Gal-3 levels remain elevated in the majority of patients despite HTx and is associated with renal dysfunction. Our findings suggest Gal-3 is a systemic rather than cardiac-specific biomarker. (J Am Coll Cardiol HF 2016;4:847-56) © 2016 by the American College of Cardiology Foundation.

Galectin (Gal)-3 (Gal-3) is a β-galactoside-binding lectin secreted by activated macrophages, which has gained interest as a novel biomarker reflecting inflammation and tissue fibrosis (1,2). Gal-3 has been shown to be associated with fibrosis and remodeling in the heart (2-5), and elevated Gal-3 levels are associated with left ventricular dysfunction and poor prognosis in patients with heart failure (HF) (6-8) and those with increased risk of incident HF and mortality in the general population (9,10).

An inverse relationship between Gal-3 level and renal function has been observed, suggesting that increased plasma Gal-3 levels may be related to renal dysfunction rather than to HF itself (6,7,11,12). However, there are no data available for the change (if any) in serum Gal-3 levels once there is improvement in cardiac function and, specifically, after heart

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ABBREVIATIONS AND ACRONYMS

AMR = antibody-mediated rejection

CAV = cardiac allograft vasculopathy

CNI = calcineurin inhibitor

CR = cellular rejection

EMB = endomyocardial biopsy

Gal = galectin

GFR = glomerular filtration rate

HF = heart failure

HTx = heart transplantation

transplantation (HTx) and reversal of the HF state. We sought to assess the significance of pre-HTx Gal-3 level on post-HTx outcomes and its role as a biomarker, given its association with cardiac fibrosis and remodeling.

The goals of this study were to evaluate the changes in serum Gal-3 levels after HTx in HF patients and to look for an association between pre- HTx elevated Gal-3 levels and post- HTx outcomes.

METHODS

PATIENT POPULATION. This was an observational study including 62 randomly selected patients who underwent HTx at our institution. Serum Gal-3 level was measured from banked plasma samples at 118 days (interquartile range [IQR]: 23 to 798 days) before and 365 days (IQR: 54 to 767 days) after HTx, using an enzyme-linked immunosorbent assay (catalog DGAL30; R&D Systems, Minneapolis, Minnesota). To categorize patients, a threshold Gal-3 value of 17.8 ng/ml was applied, based on the U.S. Food and Drug Administration cleared assay labeling for risk stratification and on published data describing significant association between Gal-3 levels >17.8 ng/ml and poor prognosis in HF patients (13). Renal function was evaluated at the time of serum Gal-3 measurement by iothalamate clearance glomerular filtration rate (GFR). Baseline characteristics pre-HTx were abstracted from the patients' electronic files. The study protocol was reviewed and approved by institutional review board at Mayo Clinic, Rochester, Minnesota.

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POST-TRANSPLANTATION FOLLOW-UP. All patients received perioperative induction therapy with rabbit antithymocyte globulin. Maintenance immunosuppression therapy post-transplantation included calcineurin inhibitor (CNI) (tacrolimus or cyclosporine), mycophenolate mofetil or azathioprine, and prednisone. In our institution, we have used the strategy of tapering steroid dose within the first 6 months after transplantation until complete withdrawal and replacing CNI-based immunosuppression with sirolimus at 6 months post HTx based on the patient's clinical status and rejection history.

Cardiac allograft function was evaluated annually by transthoracic echocardiography. Routine endomyocardial biopsies (EMB) were performed to evaluate both cellular rejection (CR) and antibody-mediated rejection (AMR), according to the 2004 and 2011 International Society for Heart and Lung Transplantation (ISHLT) working formulations, respectively (14,15). EMB were performed weekly for 4 weeks after transplantation, beginning 2 weeks after the last rabbit antithymocyte globulin dose, every 2 weeks until 2 months post transplantation, monthly from 3 to 6 months, every 3 months until the end of the first year, and yearly thereafter. EMB were also performed 10 to 15 days after any biopsy specimens that showed cellular rejection of grade 2R or higher or AMR of grade 1 or higher, and 2 weeks after any significant change in the immunosuppression regimen.

The cumulative effect of AMR and CR was calculated by rejection scores, which was the sum of EMB AMR and/or CR grading divided by the total number of biopsies performed during the first 4 years post HTx.

Cardiac allograft vasculopathy (CAV) was routinely assessed annually by coronary angiography and intravascular ultrasonography of the left anterior descending artery. CAV was defined based on the 2010 ISHLT CAV grading scale.

Functional capacity post transplantation was evaluated annually by cardiopulmonary exercise test, a symptom-limited treadmill exercise test with respiratory gas exchange analysis using a modified Naughton protocol (2-min workloads, 2 metabolic equivalent/min increments in work) as previously described (16).

MYOCARDIAL TISSUE HANDLING AND ANALYSIS. Hematoxylin-eosin-stained myocardial tissue obtained by routine EMB at 3 years post HTx was retrospectively evaluated by a cardiac pathologist (J.J.M.), who was blinded to serum Gal-3 levels, for myocyte hypertrophy and interstitial fibrosis. The degree of hypertrophy and fibrosis was assessed using a semiquantitative grading scale (0, absent; 1, mild; 2 moderate; 3, severe) and a combined score of hypertrophy and fibrosis, which was the sum of each parameter grade.

Prospective tissue sectioning and immunoperoxidase staining was performed at the Pathology Research Core (Mayo Clinic, Rochester, Minnesota), using Bond RX stain (Leica, Buffalo, Illinois). Formalin-fixed paraffin embedded tissues procured at approximately 1 year following transplantation during routine EMB were sectioned at 5 μm. Immunohistochemistry staining was performed online; tissue slides were dewaxed using Bond Dewax (Leica). Slides for Gal-3 stain were retrieved for 15 min using Epitope Retrieval 2 (EDTA, Leica), and slides for CD68 stain were retrieved for 20 min using Epitope Retrieval 2 (EDTA, Leica). The Gal-3 antibody was diluted in background-reducing diluent (Dako, Carpinteria,

California), while the CD68 antibody was diluted in Bond antibody diluent (Leica). Gal-3-stained slides were incubated in Protein Block (Dako) for 5 min. The primary antibody for Gal-3 (clone AA3A, Thermo Scientific, Grand Island, New York) was used at 1:100 dilution, and CD68 (clone PG-M1, Dako) was used at 1:200 dilution. Gal-3 slides were incubated in primary antibody for 60 min, and CD68 slides were incubated in primary antibody for 15 min.

The system used for detection of Gal-3 and CD68 was polymer refine detection system (Leica). This system includes the hydrogen peroxidase block, the post-primary and polymer reagent diaminobenzidine (DAB), and the hematoxylin stain. Immunostaining visualization was achieved by incubating slides for 10 min in DAB and DAB buffer (1:19 mixture) from the Bond polymer refine detection system. Slides were rinsed between steps with 1× Bond wash buffer (Leica). Slides were counterstained for 5 min using Schmidt hematoxylin and molecular biology-grade water (1:1 mixture), followed by several rinses in 1× Bond wash buffer and distilled water; this is not the hematoxylin provided with the refine kit. Once the immunochemistry process was completed, slides were removed from the stain and rinsed in tap water for 5 min. Slides were dehydrated in increasing concentrations of ethyl alcohol and cleared in 3 changes of xylene prior to permanent coverslipping in xylene-based medium.

Stained slides were evaluated by a cardiovascular pathologist (J.J.M.), and "hot spots" (i.e., areas containing the maximum number of macrophages) were identified. Gal-3-reactive macrophages were then quantified on the corresponding areas of the step-sectioned tissue.

Endomyocardial Gal-3 level was based on the percentage of macrophages containing Gal-3 in the myocardial tissue.

STATISTICAL ANALYSIS. Descriptive statistics are mean ± SD or n (%). Comparison of continuous measurements by Gal-3 elevated status (≤17.8 or >17.8 ng/ml) was conducted using 2-sample Student *t* tests. Changes in biomarker levels from before to after transplantation were made using the paired Student *t* test. Categorical measures were similarly compared by Gal-3 elevated status using chi-square or Fisher exact tests. Values of p shown were not corrected for multiple testing.

A Cox proportional hazard model was used to test pre-transplantation Gal-3 level as a predictor of patient mortality following transplantation.

Multivariate linear regression models were used to further examine the association between Gal-3 and GFR on $Vo_{2\ max}$, with adjustment for known confounders (age and body mass index [BMI]). All analyses were conducted using SAS version 9.3 software (SAS, Cary, North Carolina).

RESULTS

STUDY POPULATION. The study cohort consisted of 62 patients who underwent heart (n = 50), heart-kidney (n = 6), and heart-liver (n = 6) transplantation. Causes of HF included ischemic heart disease (13 [21%]), dilated cardiomyopathy (16 [26%]), restrictive cardiomyopathy (23 [37%]), valvular heart

TABLE 1 Baseline Characteristics According to Pre-Transplantation Gal-3 Levels

	Gal-3 <17.8 ng/ml (n = 27)	Gal-3 ≥17.8 ng/ml (n = 35)	p Value
Age, yrs	50 ± 11	56 ± 10	0.052
Males	25 (93)	22 (63)	0.007
BMI, kg/m^2	26 ± 4	28 ± 4	0.230
DM	3 (11)	8 (23)	0.321
HTN	4 (15)	4 (11)	0.719
Creatinine	1.23 ± 0.5	1.36 ± 0.5	0.224
GFR	72 ± 23	59 ± 20	0.007
COPD	1 (4)	3 (9)	0.626
Atrial fibrillation	7 (26)	15 (43)	0.167
Smoking history	9 (33)	17 (49)	0.228
Alcohol abuse history	4 (15)	2 (6)	0.390
Inotropic support pre-transplant	13 (48)	24 (69)	0.104
VAD support pre-transplant	6 (22)	4 (12)	0.315
6-min walk distance pre-transplant, m	403 ± 116	375 ± 118	0.474
Peak Vo_2, l/min/m^2	14 ± 6	13 ± 4	0.724
NT-proBNP	2,551 ± 2,507	3,150 ± 2,591	0.204
ST2 level	37.8 ± 12.5	60 ± 63	0.229
PRA class I, %	7 ± 11	4 ± 8	0.573
PRA class II, %	14 ± 21	6 ± 12	0.092
Positive DSA	6 (43)	6 (32)	0.506
Echocardiographic parameters			
EF, %	29 ± 20	31 ± 17	0.218
LVEDD, mm	60 ± 16	61 ± 16	0.699
LV mass index	153 ± 68	130 ± 59	0.239
LA volume index	55 ± 20	69 ± 29	0.151
E/A ratio	2.0 ± 1.2	2.3 ± 1.3	0.311
E/e′ ratio	17 ± 9	23 ± 10	0.018
Deceleration time (ms)	178 ± 74	154 ± 34	0.308
RV function grade	2 ± 1	1 ± 1	0.098
Hemodynamic parameters			
Mean RA pressure, mm Hg	13 ± 6	14 ± 7	0.672
Mean PA pressure, mm Hg	30 ± 11	33 ± 10	0.139
Wedge pressure, mm Hg	20 ± 8	22 ± 8	0.224
Cardiac output, l/min	4.1 ± 1.3	4.3 ± 1.2	0.619
Cardiac index, l/min/m^2	2.1 ± 0.6	2.3 ± 0.7	0.518
Pulmonary vascular resistance, WU	3 ± 2	3 ± 2	0.590

Values are mean ± SD or n (%).

BMI = body mass index; COPD = chronic obstructive pulmonary disease; DM = diabetes mellitus; DSA = donor specific antibodies; EF = ejection fraction; Gal = galectin; GFR = glomerular filtration rate; HTN = hypertension; LA = left atrium; LV = left ventricle; LVEDD = left ventricular end diastolic diameter; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PA = pulmonary artery; PRA = panel reactive antibody; RA = right atrium; RV = right ventricle; VAD = ventricular assist device; Vo_2 = oxygen consumption; WU = wood units.

disease (3 [5%]), congenital heart disease (5 [8%]), and arrhythmogenic cardiomyopathy (2 [3%]). Patients were divided into 2 groups based on pre-HTx Gal-3 levels: those with elevated Gal-3 levels (≥17.8 ng/ml; n = 35) and those with normal Gal-3 levels (<17.8 ng/ml; n = 27). Baseline and post-transplantation characteristics are described in Table 1 and Table 2, respectively.

TABLE 2 Post-Transplantation Parameters According to Pre-Transplantation Gal-3 Levels

	Gal-3 <17.8 ng/ml (n = 27)	Gal-3 ≥17.8 ng/ml (n = 35)	p Value
Total waiting time, days	271 ± 231	297 ± 277	0.681
Allograft ischemic time, min	180 ± 65	183 ± 62	0.755
Recipient bypass time, min	96 ± 82	91 ± 78	0.976
Post-transplant DM	8 (30)	12 (34)	0.697
Post-transplant HTN	20 (74)	30 (86)	0.250
Post-transplant dyslipidemia	24 (89)	31 (89)	1.000
Post-transplant Gal-3 levels	16.1 ± 6.0	22.7 ± 7.2	0.025
Post-transplant ST2 levels	42.1 ± 19.0	38.2 ± 12.3	0.715
Post-transplant NT-proBNP levels	607 ± 513	697 ± 596	0.556
Immunosuppression therapy at baseline			
MMF therapy	21 (78)	29 (83)	0.616
Azathioprine therapy	6 (22)	6 (17)	
Cyclosporine therapy	20 (74)	26 (74)	0.985
Tacrolimus therapy	7 (26)	9 (26)	
Immunosuppression therapy at 1 yr			
Tacrolimus therapy	5 (19)	9 (26)	0.502
Tacrolimus dose/level	0.82 ± 0.41	0.97 ± 0.53	0.724
Cyclosporine therapy	17 (63)	16 (46)	0.177
Cyclosporine dose/level	1.68 ± 0.75	1.87 ± 1.02	0.759
Sirolimus therapy	10 (37)	13 (37)	0.993
Sirolimus dose/level	0.45 ± 0.49	0.35 ± 0.26	0.862
Prednisone therapy	26 (96)	34 (97)	0.852
Cardiac allograft function			
EF at 1 yrs	61 ± 8	61 ± 7	0.238
EF at 2 yrs	62 ± 10	61 ± 8	0.301
EF at 3 yrs	61 ± 6	63 ± 7	0.133
Cardiac allograft vasculopathy (≥moderate degree)			
1 yr	1 (4.2)	4 (12.1)	0.385
2 yrs	1 (4.8)	4 (12.9)	0.637
3 yrs	3 (15.0)	5 (16.7)	1.000
4 yrs	6 (33.3)	9 (34.6)	1.000
Cellular rejection score ≥0.3			
1 yr	13 (21.0)	19 (30.7)	0.632
2 yrs	13 (22.0)	16 (27.1)	0.708
3 yrs	11 (21.2)	16 (51.6)	0.957
4 yrs	8 (24.2)	11 (33.3)	0.424
Antibody-mediated rejection score			
1 yr	0.03 ± 0.06	0.05 ± 0.16	0.903
2 yrs	0.04 ± 0.07	0.05 ± 0.15	0.637
3 yrs	0.03 ± 0.20	0.04 ± 0.11	0.711
4 yrs	0.03 ± 0.06	0.06 ± 0.12	1.000
Mortality	5 (18.5)	3 (8.6)	0.238

Values are mean ± SD or n (%).
MMF = mycophenolate mofetil; other abbreviations as in Table 1.

POST-TRANSPLANTATION IMMUNOSUPPRESSION THERAPY. Maintenance immunosuppression therapy with CNI was replaced with sirolimus in 23 patients (37%) during the follow-up period. There were no significant differences between the immunosuppression therapy received by patients with elevated Gal-3 and that received by patients with normal Gal-3 levels at baseline and at 1 year post-HTx (Table 2).

CIRCULATING Gal-3 LEVELS BEFORE AND AFTER HEART TRANSPLANTATION. Mean Gal-3 levels measured before and after HTx among the study cohort were 20.3 ± 8.6 ng/ml and 19.8 ± 7.4 ng/ml, respectively. Gal-3 levels were elevated in 35 patients before HTx and remained elevated post HTx in 26 of them. The mean post-HTx Gal-3 level was higher among patients with elevated pre-HTx Gal-3 than in those with normal pre-HTx levels (22.7 ± 7.2 ng/ml vs. 16.1 ± 6.0 ng/ml, respectively; p = 0.025) (Figure 1A).

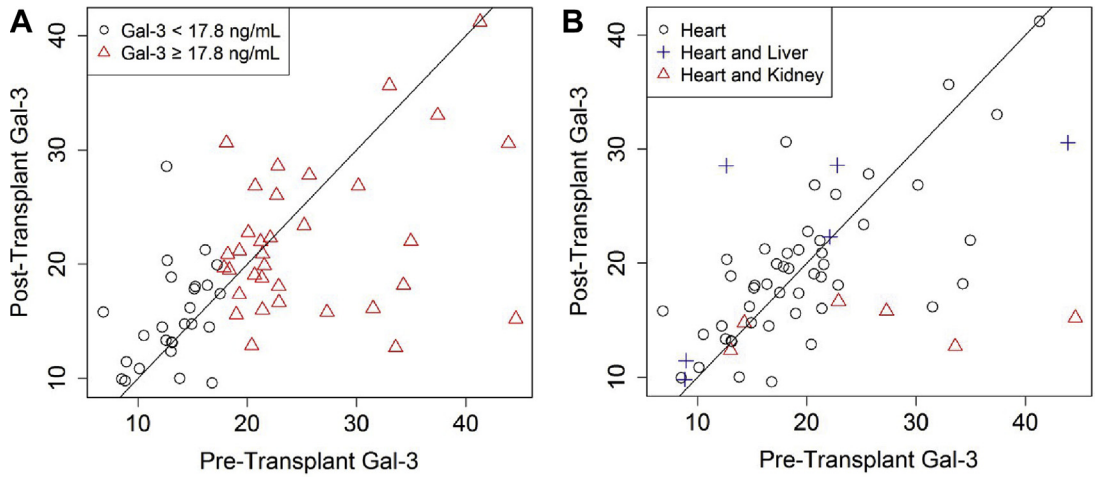
Gal-3 levels did not change significantly before and after HTx among those who received heart transplants alone (19.6 ± 7.4 vs. 20.2 ± 7.4, respectively; paired difference: 0.6 [6.7]; p = 0.56) or those who received heart and liver transplants (19.9 ± 13.3 vs. 21.9 ± 9.2, respectively; paired difference: 2.0 [9.5]; p = 0.62), but there was a trend toward lower Gal-3 levels in patients who underwent heart and kidney transplantation (25.9 ± 12.0 vs. 14.6 ± 1.7, respectively; paired difference: −11.4 [11.8]; p = 0.06) (Figure 1B).

Two other heart failure biomarkers (ST2 and N-terminal pro-B-type natriuretic peptide [NT-proBNP]) were measured at the same time point Gal-3 measurements were made before and after HTx. The median ST2 and NT-proBNP levels were significantly reduced post transplantation (ST2: 38.4 ng/dl [median levels: 30.2 to 46.1 ng/dl] vs. 34.8 ng/dl [median levels: 27.2 to 39.3 ng/dl], respectively; p = 0.05; and NT-proBNP: 2,207 pg/ml [IQR: 953 to 3,353 pg/ml] vs. 503 pg/ml [IQR: 266 to 988 pg/ml], respectively; p < 0.001). There was no association between pre-transplantation Gal-3 levels and these biomarkers measured before or after HTx (Tables 1 and 2).

CIRCULATING Gal-3 LEVELS AND RENAL FUNCTION. GFR was found to be significantly lower in patients with elevated Gal-3 levels than in patients with normal levels (pre-HTx levels: 51 ± 22 ml/min vs. 73 ± 22 ml/min, respectively; p = 0.0002; post-HTx levels: 59 ± 22 ml/min vs. 73 ± 24 ml/min, respectively; p = 0.010) (Figure 2). There was also an inverse correlation between the changes in Gal-3 levels and GFR before and after HTx (r = −0.35; p = 0.007) (Figures 3A to 3C).

SERUM AND TISSUE Gal-3 CORRELATION. There was no correlation between the serum Gal-3 levels before

FIGURE 1 Gal-3 Levels Pre- and Post-Transplant According to Pre-Transplant Gal-3 Levels

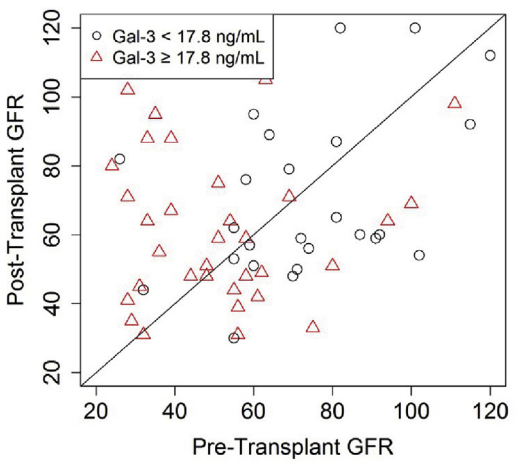


(A) The mean post-HTx Gal-3 level was higher among patients with elevated pre-HTx Gal-3 than among those with normal pre-HTx levels. **(B)** Gal-3 levels before and after transplantation are shown by transplant type. No significant changes in Gal-3 levels were observed before and after HTx among recipients of heart-alone or heart-and-liver transplantation. A trend toward lower Gal-3 levels was evident after heart and kidney transplantation. Gal = galectin; HTx = heart transplantation.

or after HTx and the Gal-3 staining measured in the myocardial tissue at 1 year post-transplantation ($r = 0.15$; $p = 0.31$).

Elevated Gal-3 levels before or after HTx were associated with neither myocyte hypertrophy nor interstitial fibrosis within the cardiac allograft at 3 years post HTx (Table 3). There was also no association between the other HF biomarkers (ST2 and NT-proBNP) and myocardial tissue remodeling ($p = 0.58$ and $p = 0.69$, respectively).

FIGURE 2 GFR Pre and Post Transplantation According to Pre-Transplant Gal-3 Levels



Glomerular filtration rate (GFR) was significantly lower in patients with elevated galectin (Gal)-3 levels than in those with normal levels.

Pre-HTx Gal-3 LEVELS AND POST-HTx OUTCOMES. The mean follow-up post HTx was 81 ± 34 months. There was a significant association between elevated pre-HTx Gal-3 levels and reduced functional capacity evaluated by cardiopulmonary exercise test results compared to results in patients with normal pre-HTx Gal-3 levels ($p < 0.05$) (Table 4, Figure 4).

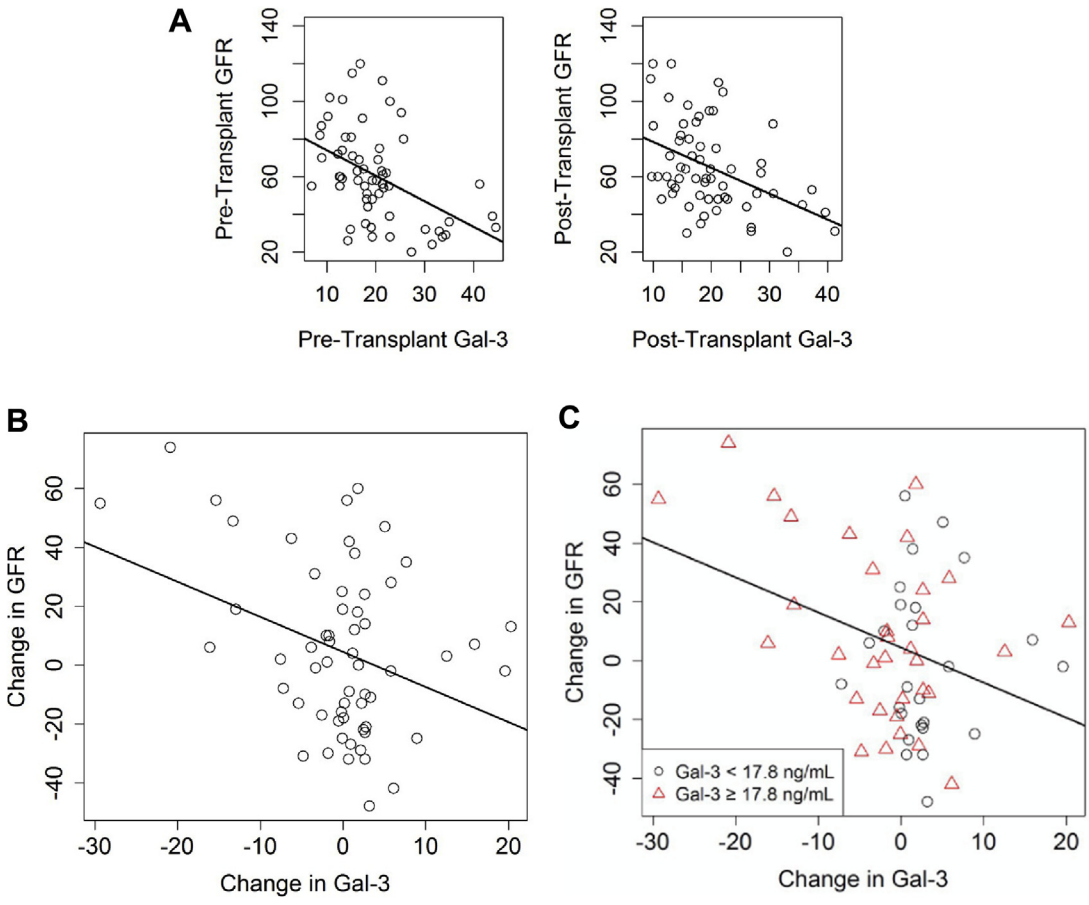
There was no significant association between pre-HTx elevated Gal-3 levels and other post-transplantation outcomes including cardiac allograft function, CAV, and rejection episodes (Table 2).

Multivariate analysis was performed to evaluate the association between pre-HTx Gal-3 levels and peak Vo_2 at 2 and 3 years post HTx (Online Tables 1 and 2). After adjustment for age, BMI, and pre-HTx GFR, variables that have previously been reported to affect functional capacity post transplantation, pre-transplantation, Gal-3 level was no longer associated with reduced functional capacity post transplantation. Age, BMI, and GFR pre-transplantation were found to be associated with reduced peak Vo_2 post HTx (Online Tables 1 and 2).

DISCUSSION

This is the first study to evaluate changes in serum Gal-3 levels, a biomarker of poor prognosis and adverse remodeling, in HF patients after "reversal" of

FIGURE 3 Inverse Correlation Between the Change in Gal-3 Levels and GFR Before and After HTx



(A) Scatter plot of GFR and Gal-3 pre and post transplantation with regression line. **(B)** Scatter plot of the change in GFR versus the change in Gal-3 with regression line. **(C)** Scatter plot of the change in GFR versus the change in Gal-3 with regression line and different symbols to represent pre-transplantation Gal-3 grouping. Abbreviations as in Figures 1 and 2.

TABLE 3 Gal-3 and Cardiac Allograft Hypertrophy and Fibrosis

	Pre-HTx Gal-3			Post-HTx Gal-3		
	≤17.8 ng/ml (n = 19)	>17.8 ng/ml (n = 25)	p Value	≤17.8 ng/ml (n = 19)	>17.8 ng/ml (n = 25)	p Value
Myocyte hypertrophy						
Absent/mild	13 (68)	18 (72)	0.80	14 (74)	17 (68)	0.68
Moderate/severe	6 (32)	7 (28)		5 (26)	8 (32)	
Pericellular fibrosis						
Absent/mild	16 (84)	21 (84)	1.00	14 (74)	23 (92)	0.21
Moderate/severe	3 (16)	4 (16)		5 (26)	2 (8)	
Combined hypertrophy and fibrosis score						
Combined score (0–2)	11 (58)	17 (68)	0.49	11 (58)	17 (68)	0.49
Combined score (3–6)	8 (42)	8 (32)		8 (42)	8 (32)	

HTx = heart transplantation; other abbreviations as in Table 1.

the HF state with HTx and its role in prediction of outcomes in the HTx patient population. The first major finding of this study was that serum Gal-3 levels remains elevated in most HF patients despite HTx; second, there is a strong inverse correlation between Gal-3 levels and renal function before and after HTx; third, there was no correlation between Gal-3 level in the serum and that in myocardial tissue post HTx; fourth, higher Gal-3 levels were associated with neither myocyte hypertrophy nor interstitial fibrosis post HTx; and fifth; elevated pre-HTx Gal-3 levels are associated with poor exercise capacity post HTx, but the association was not significant after adjustment for age, BMI, and renal function. Our findings suggest that Gal-3 is a systemic rather than a tissue-specific

TABLE 4 Post-Transplantation Cardiopulmonary Stress Test Parameters According to Pre-Transplantation Gal-3 Levels

	Pre-Gal-3 <17.8 ng/ml	Pre-Gal-3 ≥17.8 ng/ml	p Value
Peak heart rate			
1 yr	133 ± 20	132 ± 15	0.76
2 yrs	140 ± 19	134 ± 22	0.54
3 yrs	147 ± 24	133 ± 19	0.07
Systolic blood pressure response			
1 yr	27 ± 32	35 ± 18	0.68
2 yrs	33 ± 24	36 ± 18	0.31
3 yrs	32 ± 22	34 ± 21	0.64
Exercise duration, min			
1 yr	8 ± 2	7 ± 1	0.02
2 yrs	9 ± 2	7 ± 2	0.03
3 yrs	9 ± 2	7 ± 2	0.01
METS			
1 yr	5.6 ± 1.5	4.9 ± 1.0	0.11
2 yrs	6.2 ± 1.4	4.8 ± 1.1	0.01
3 yrs	6.8 ± 1.9	4.9 ± 1.4	0.01
Peak Vo_2			
1 yr	20 ± 5	17 ± 4	0.11
2 yrs	22 ± 5	17 ± 4	0.01
3 yrs	24 ± 7	17 ± 5	0.01
RER			
1 yr	1.2 ± 0.1	1.2 ± 0.1	0.74
2 yrs	1.3 ± 0.1	1.2 ± 0.1	0.29
3 yrs	1.3 ± 0.1	1.2 ± 0.1	0.45
VE/VCo_2			
1 yr	31 ± 3	34 ± 5	0.04
2 yrs	31 ± 3	33 ± 4	0.03
3 yrs	30 ± 3	33 ± 4	0.01
Breathing reserve			
1 yr	51 ± 13	46 ± 14	0.23
2 yrs	45 ± 11	44 ± 15	0.65
3 yrs	42 ± 13	47 ± 14	0.31
Anaerobic threshold			
1 yr	1,092 ± 296	1,060 ± 299	0.72
2 yrs	1,218 ± 341	1,032 ± 289	0.09
3 yrs	1,257 ± 413	954 ± 338	0.03

Values are mean ± SD.

METS = metabolic equivalents; RER = respiratory exchange ratio; VE/VCo_2 = ventilation/Co_2 output ratio; Vo_2 = oxygen consumption; y = year post transplantation.

biomarker and that the ability of Gal-3 to predict outcomes in HF and in HTx may reflect renal impairment in both of these patient populations.

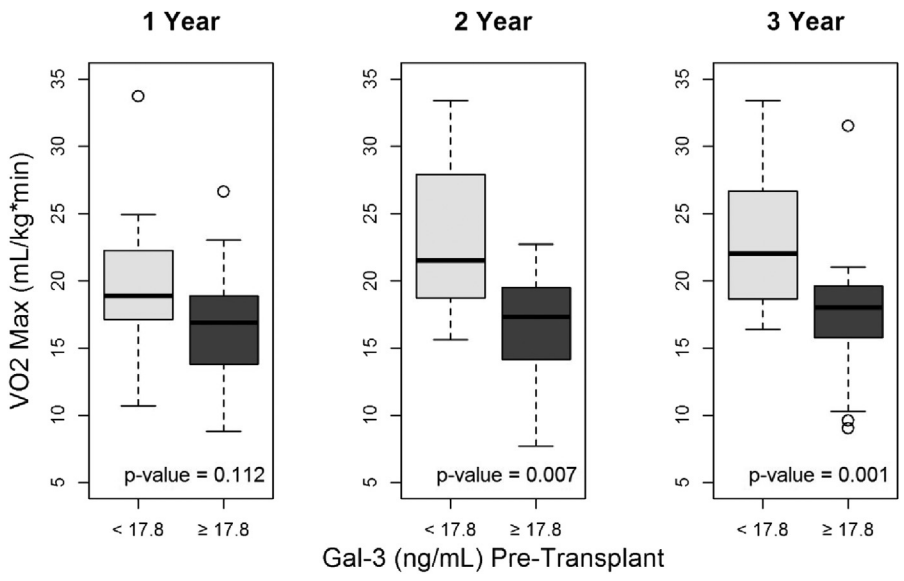
ASSOCIATION OF Gal-3 LEVELS AND MYOCARDIAL FIBROSIS. Gal-3 has been shown to be up-regulated in various inflammatory disorders affecting the liver, lung, and kidney (4). It also plays an important role in the regulation of pro-fibrotic pathways in the heart (3–5); therefore, clinical data support the notion that serum Gal-3 levels reflect myocardial fibrosis (17). However other studies have produced conflicting results. Frunza el al. (18) demonstrated that, despite up-regulation of Gal-3 in pressure-overloaded myocardium, there was no significant association between Gal-3 and systolic or diastolic dysfunction or myocardial fibrosis, suggesting Gal-3 does not play a crucial role in the pathogenesis of the fibrotic cardiomyopathy associated with pressure overload. Another study supporting this concept by Abou Ezzeddine et al. (19) demonstrated that Gal-3 levels were not independently associated with severity of heart failure with preserved ejection fraction.

Myocardial fibrosis has been described in serial EMB results of cardiac allografts (20) and was found to be an important contributor to the development of restrictive cardiac physiology among HTx recipients (21). We hypothesized that higher serum Gal-3 levels, especially if persistent, may identify patients with advanced replacement myocardial fibrosis with its clinical consequences post HTx. However, we found no interaction between elevated serum Gal-3 levels and myocardial tissue fibrosis or hypertrophy post HTx, perhaps due to lack of correlation between serum and myocardial tissue Gal-3 post-HTx. Differences between serum Gal-3 levels and myocardial tissue expression have been previously reported by Beiras-Fernandez et al. (22). Serum levels were measured and expression of myocardial Gal-3 was assessed by real-time PCR and immunohistochemistry in patients with end-stage HF undergoing HTx and were compared to healthy controls. Although serum levels of Gal-3 were significantly higher in the end-stage HF patients than in the healthy controls, the expression levels of Gal-3 in the myocardial tissue were similar between the groups (22).

TEMPORAL CHANGES IN SERUM Gal-3 LEVELS IN HF AND HTx. Our study demonstrates that Gal-3 levels may remain persistently elevated even after replacement of the diseased myocardium and reversal of the HF state with HTx.

Serial increase in Gal-3 levels was found to be associated with higher risk for subsequent HF morbidity and mortality (23–25); however, Anand et al. (24) found, by examining changes in Gal-3 levels over 12 months in HF patients who had participated in the Val-HeFT (Valsartan Heart Failure Trial), these changes were associated with changes in GFR and not reverse remodeling. Other studies have not demonstrated a change in Gal-3 levels with optimization of HF medications (25), cardiac resynchronization therapy (26), or mechanical circulatory support (27). Our study supports the findings of these studies that serum Gal-3 level is unlikely to be a cardiac-specific biomarker and is not associated with an improvement in cardiac function but is more likely to be related to renal function.

FIGURE 4 Peak Vo_2 Post Transplantation According To Pre-Transplant Gal-3 Levels



Significant association between patients with elevated pre-HTx Gal-3 levels and reduced functional capacity at 2 and 3 years post transplantation and those who had normal pre-HTx Gal-3 levels. **(Light gray boxes)** Normal pre-transplantation Gal-3 levels (<17.8); **(Dark gray boxes)** elevated pre-transplantation Ga-3 levels (>17.8). Abbreviations as in Figure 1.

ASSOCIATION OF Gal-3 LEVELS WITH RENAL FUNCTION. An inverse relationship between serum Gal-3 and renal function has been observed in patients with HF (6,7,12), implying that increased Gal-3 levels in HF might be due to renal dysfunction and that the ability of Gal-3 to predict outcomes in HF might reflect, at least in part, the consequences of renal impairment (5). In addition, the correlation between Gal-3 levels and GFR in patients with renal failure and those without HF was comparable to the group of HF patients (11). Furthermore, the incremental value of Gal-3 in addition to established risk factors for mortality in chronic HF patients (including age and renal function) was insignificant, unlike ST2, a different myocardial fibrosis biomarker (28).

By demonstrating persistence of elevated Gal-3 levels despite correction of the HF state and its inverse relationship to renal function, the current study supports the known association between elevated Gal-3 levels and renal dysfunction. We also extend this observation by demonstrating for the first time the persistent association between pre-HTx elevated Gal-3 levels and renal dysfunction post HTx. Conversely, the decrease in Gal-3 levels observed after combined heart and kidney transplantation compared to those after heart alone or combined heart and liver transplantation supports the notion that the source of Gal-3 may be the kidney itself, as previously suggested by our group, using immunohistochemical staining of Gal-3 in renal distal tubular epithelial cells (29).

ASSOCIATION OF Gal-3 LEVELS WITH FUNCTIONAL CAPACITY POST HTx. Higher serum Gal-3 levels have been shown in previous studies to be associated with reduced functional capacity in patients with heart failure with reduced ejection fraction, but no adjustment for renal function was made in these studies (30). In patients with heart failure with preserved ejection fraction, an association was demonstrated between elevated Gal-3 levels and lower peak Vo_2 values and 6-min walk distances, but this association, similar to the findings in our study, was no longer significant after adjustment for renal function (19).

There are multiple factors that could affect exercise performance after HTx. Our findings confirm previously published data showing that obesity and increased recipient age may be associated with impaired exercise capacity post HTx (31,32). Chronotropic incompetence has been reported as a mechanism for reduced exercise tolerance in HTx recipients (33); however, age, BMI, and chronotropic and blood pressure responses to exercise among patients with elevated Gal-3 levels were not significantly different from those of patients without elevated Gal-3 levels in our study.

Our group has demonstrated that left ventricular hypertrophy after HTx is associated with exercise intolerance (32) and that it has been shown to be associated with subsequent development of CAV and mortality (34). We therefore examined biopsy specimens for myocyte hypertrophy and interstitial fibrosis, histological findings that have been associated with elevated Gal-3 at 3 years post HTx between the high- and low-Gal-3 groups. There were no significant differences in myocyte hypertrophy or interstitial fibrosis between the 2 groups that could have explained the exercise intolerance observed in the high-Gal-3 group. Furthermore there were no differences in cardiac allograft function, CAV, or allograft rejection between the 2 groups.

Renal dysfunction is common after HTx and can be a major source of morbidity and mortality in this population (35–37). Our study confirms the association shown in prior studies between reduced renal function and reduced exercise capacity post HTx (38); however, because this association is accompanied with elevated Gal-3 levels, whether Gal-3 mediates this effect is unknown.

Another mechanism that may account for exercise intolerance is peripheral vascular endothelial dysfunction, which has been shown to correlate with reduced exercise capacity in cardiac transplant recipients independent of age or left ventricular ejection fraction (33). Gal-3 plays an important role in the regulation of proinflammatory pathways in the heart (3–5), and other studies suggest that increased Gal-3 levels observed in cardiovascular pathologies originate at least partially from endothelial cells and potentially contribute to vascular inflammation (39). The role of Gal-3 in the pathophysiology of exercise intolerance due to its role as a mediator of endothelial dysfunction must be investigated further in future studies.

STUDY LIMITATIONS. We recognize several limitations in our study. First, this was a retrospective study conducted in a single large referral center, resulting in possible patient selection bias, and the cause of HF in the study population may not reflect that in the standard HF population. Second, the relatively small cohort size may lack statistical power to identify association between Gal-3 levels and post HTx outcomes and to identify significant changes in Gal-3 levels post transplantation between recipients with heart-kidney transplantation versus those who underwent HTx alone. Third, all stained tissue samples were read by one pathologist, and hence, concordance in histological interpretation was not measured.

CONCLUSIONS

The persistently elevated circulating Gal-3 in HF patients despite HTx, the strong inverse correlation between Gal-3 levels and renal function, and the lack of association between serum levels and myocardial tissue staining post HTx validate findings from other studies that Gal-3 is a systemic rather than a cardiac-specific biomarker. Elevated pre-HTx Gal-3 levels were associated with poor exercise capacity post HTx, but the association was eliminated after adjustment for age, BMI, and renal function validating findings from other studies that Gal-3 is strongly correlated with renal function rather than the HF state. However, whether Gal-3 associated with renal dysfunction plays a role in the pathophysiology of post-HTx complications such as exercise intolerance remains to be proven.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Circulating Gal-3 levels, a biomarker for HF, may remain persistently elevated post HTx despite reversal of the HF state and is inversely associated with renal function post transplantation.

TRANSLATIONAL OUTLOOK: Circulating Gal-3 levels, a known prognostic biomarker for HF, remain persistently elevated despite correction of the HF state with HTx and therefore is more likely a systemic biomarker rather than a cardiac-specific biomarker. There appears to be an inverse relationship between Gal-3 and renal function post HTx, including concomitant renal transplantation that suggests that the source of Gal-3 may be the kidney itself.

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