Original Article

Plasma and Cardiac Galectin-3 in Patients With Heart Failure Reflects Both Inflammation and Fibrosis
Implications for Its Use as a Biomarker

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Background—Galectin (Gal)-3 is a β-galactoside-binding lectin and currently intensely studied as a biomarker in heart failure. Gal-3 also exerts proinflammatory effects, at least in extracardiac tissues. Objective of this study was to characterize the relationship of plasma and myocardial Gal-3 levels with cardiac fibrosis and inflammation in patients with nonischemic dilated cardiomyopathy and inflammatory cardiomyopathy (iCMP).

Methods and Results—Endomyocardial biopsies and blood samples were obtained from patients with newly diagnosed cardiomyopathy and clinical suspicion of myocarditis. According to histopathologic findings, patients were classified as having dilated cardiomyopathy (n=40) or iCMP (n=75). Cardiac fibrosis was assessed histologically on endomyocardial biopsy sections. In patients with iCMP, myocardial Gal-3 expression significantly correlated with inflammatory cell count on endomyocardial biopsy ($r=0.56$; $P<0.05$). In contrast, an inverse association was observed between myocardial Gal-3 expression and cardiac fibrosis in patients with iCMP ($r=-0.59$; $P<0.05$). In patients with dilated cardiomyopathy, myocardial Gal-3 expression correlated with cardiac fibrosis on left ventricular biopsy ($P=0.63$; $P<0.01$). Of note, in both groups, plasma Gal-3 levels did not correlate with myocardial Gal-3 levels or left ventricular fibrosis, whereas a positive correlation between plasma Gal-3 levels and inflammatory cell count on endomyocardial biopsy was observed in patients with iCMP.

Conclusions—The present study suggests that myocardial Gal-3 can be considered as a possible marker for both cardiac inflammation and fibrosis, depending on the pathogenesis of heart failure. However, circulating concentrations of Gal-3 do not seem to reflect endomyocardial Gal-3 levels or cardiac fibrosis. (*Circ Heart Fail*. 2017;10:e003804. DOI: 10.1161/CIRCHEARTFAILURE.116.003804.)

Key Words: biomarker ■ galectin-3 ■ fibrosis ■ heart failure ■ inflammation ■ inflammatory cardiomyopathy

Dysregulation of myocardial extracellular matrix remodeling and cardiac fibrosis is a key pathological feature of essentially any cardiovascular disease.[1,2] Myocardial fibrosis contributes to the progression of heart failure and is linked to poor outcome in patients with cardiovascular disease.[3] Consequently, predictors of myocardial fibrosis have been suggested as valuable markers of disease progression or measures of therapeutic success.[3] Plasma or serum levels of galectin-3 (Gal-3), a member of the carbohydrate-binding protein family of lectins, are currently studied as a potential novel biomarker for cardiac fibrosis and adverse cardiac remodeling in heart failure.[3,4] Experimental studies have implicated that Gal-3 is secreted by activated macrophages and mediates profibrotic processes in rodent models of heart failure.[5,6] Gal-3 has been linked to enhanced myofibroblast proliferation, collagen production, macrophage infiltration, and cardiac hypertrophy, at least in part via stimulation of the transforming growth factor-β/SMAD3 signaling pathway.[5–7]

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However, whether circulating levels of Gal-3 indeed reflect myocardial Gal-3 levels and cardiac fibrosis in a given patient with heart failure remains to be verified. The utility of Gal-3 as a biomarker for fibrosis might also be complicated by the fact that Gal-3 has been suggested as a regulator of acute and chronic inflammation.[8] Emerging evidence suggests that Gal-3 can modulate adaptive immune responses by

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influencing T cell homeostasis and B cell function.[4] Notably, markers of inflammation on endomyocardial biopsies (EMBs) are observed in ≤40% of patients with dilated cardiomyopathy (DCM) and symptomatic heart failure, despite standard pharmacological treatment.[9] Therefore, Gal-3 levels may be altered by different pathological factors in patients with heart failure, depending on the pathogenesis of the underlying cardiomyopathy.

Previous studies reported contradicting results on the association between plasma and myocardial levels of Gal-3 and their role as estimates for fibrosis.[10–12] However, these studies were limited by the insufficient sample size[10] and did not assess myocardial inflammation as an alternative contributor to Gal-3 levels.[11,12]

Therefore, we sought to assess the following in subjects with nonischemic, noninflammatory DCM, primarily characterized by replacement fibrosis, and subjects with inflammatory cardiomyopathy (iCMP), characterized by varying degrees of fibrosis and inflammation: (1) the association of plasma and myocardial Gal-3 levels with myocardial fibrosis and inflammation; (2) the suitability of plasma Gal-3 levels as a reflect of myocardial Gal-3 levels.

Methods

Patient Sample

In this prospective cohort study, patients with heart failure because of an unexplained cardiomyopathy or clinically suspected myocarditis were included if they fulfilled one of the following criteria: (1) new onset or persisting symptoms suggestive of heart failure; and (2) suspicion of myocarditis according to previously published criteria.[13–16] In addition, relevant coronary artery disease had to be ruled out on selective angiography. Patients with contraindication to cardiac catheterization or EMB were excluded. Patients were divided into 2 groups according to immunohistological findings on EMB to separate patients with nonischemic, noninflammatory DCM (<14 leucocytes/mm^2) and patients with iCMP (≥14 leucocytes/mm^2, including ≥7 cells/mm^2 CD3-positive T-lymphocytes, CD68-positive macrophages, in addition to enhanced human leukocyte antigen class II expression in professional antigen-presenting immune cells), in accordance with recent recommendations of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases.[13] Blood sampling, echocardiography, and cardiac magnetic resonance imaging were performed at study entry. All subjects gave written informed consent prior to inclusion in the study, and the study was approved by the local ethics committee.

Coronary Angiography and EMBs

Significant coronary artery disease (defined as stenosis >50% of vessel diameter) was excluded by coronary angiography. For EMB sampling, a myocardial biopsy forceps (Teleflex Medical Tuttlingen GmbH, Tuttlingen, Germany) was used. Five to 6 EMBs were taken from the left and right ventricle under fluoroscopic guidance. EMBs were taken from different locations within the ventricles. For each ventricle, 2 to 3 biopsy specimens were fixed in 4% buffered formaldehyde for immunohistology. Two tissue samples were fixed in RNAlater (Ambion Inc., Foster City) for detection of viral genomes by nested real-time reverse transcriptase polymerase chain reaction, and 1 biopsy specimen was used for RNA isolation to quantify Gal-3 levels.

EMB Analyses

All histological and immunohistological analyses diagnosing myocardial inflammation were performed at the Department of Molecular Pathology, University Hospital Tuebingen (Tuebingen, Germany), as previously published.[15,17,18] Paraffin-embedded EMBs were stained with Masson's trichrome, reflecting myocyte necrosis, as well as interstitial fibrosis, and analyzed by light microscopy, as published.[17,19] Fibrotic and artifact areas were digitally marked on stained EMB sections. The percentage area of fibrosis in the section was evaluated by dividing the sum of the fibrotic areas of the section by that of the total tissue area as described previously.[20]

Quantification of Gal-3 mRNA Expression in EMBs

Total RNA was isolated from EMBs using the RNeasy kit (Qiagen, Hilden, Germany) according to manufacturer's instruction. In brief, EMBs were treated with an RNA stabilization reagent and homogenized using a TissueRuptor. Hundred nanogram of total RNA was reverse transcribed into cDNA using random hexamers and Sensiscript reverse transcriptase (Qiagen). An aliquot of the cDNA was used for quantitative reverse transcriptase polymerase chain reaction applying the Light Cycler system (Roche Diagnostics Inc, Mannheim, Germany). The expression of Gal-3 was normalized to the respective TATA-binding protein levels in EMBs. The following primers were used: Gal-3 (sense) 5′-ggCCACTgATTgTgCCTTAT, Gal-3 (antisense) 5′-TCTTTCTTCCCTTCCCCAgT, TATA-binding protein (sense) 5′-gAgAgCCACgAACCACgg, and TATA-binding protein (antisense) 5′-ACATCACAgCTCCCCACCAT.

Quantification of Gal-3 Protein Expression in EMBs

Protein expression of Gal-3 in EMBs from patients with DCM and iCMP was analyzed by immunohistochemistry. In brief, paraffin-embedded EMB sections were deparaffinized in xylol/ethanol and rehydrated. After heat-induced epitope retrieval (10 mmol/L Citrate, pH 6.0), EMB sections were blocked and incubated with a rabbit polyclonal anti-Gal-3 antibody (Abcam, Cambridge, United Kingdom), followed by incubation with a goat anti-rabbit Alexa fluor 488 secondary antibody (Thermo Fisher Scientific, Waltham, MA) and Hoechst 33342 staining.

Quantification of Gal-3 Levels in Plasma

Fasting venous blood samples were drawn from patients with DCM and iCMP on cardiac catheterization immediately after puncture of the femoral vein. EDTA-containing plasma tubes were immediately centrifuged, and plasma aliquots stored at −80°C until final analysis without any freeze-thaw cycle. Gal-3 concentration in plasma was analyzed in duplicate by a commercially available ELISA kit (Norcross, GA). Immunofluorescence was detected on a microplate reader (Fluostar Optima, BMG Labtech), and Gal-3 concentration in plasma samples was calculated according to manufacturer's instructions. Intra- und interassay coefficients of variation were <10% and <12%, respectively. The recovery of analyte from the assay was 113.2% on average (range 103%–128%). All samples analyzed in the present study were above the detection limit.

Statistical Analysis

Kolmogorov–Smirnov testing was performed for assessment of data distribution and variance homogeneity. Continuous variables of the patient sample are presented as mean and standard deviation if normally distributed, or as median and interquartile range if non-normally distributed. Categorical variables are expressed as frequencies and percentages. Continuous variables were compared between groups using Student's t test or Mann–Whitney U test as appropriate if equal variances were present; categorical variables were compared using the Chi-squared test. An unequal variance t test (Welch test) was applied if variance heterogeneity was present. Myocardial and plasma Gal-3 levels, myocardial fibrosis, and inflammation are presented as boxplots showing the median, interquartile range, and variation. The association between myocardial Gal-3 levels and myocardial fibrosis or inflammation was assessed by Spearman correlation analysis. Two-sided P values <0.05 were considered statistically significant for all statistical procedures used. All statistical analyses were performed by SPSS version 21 (SPSS, Inc, Chicago, IL).

Results

Patient Characteristics

In total, 40 patients with nonischemic DCM and 75 patients with iCMP were included in the present study. The characteristics of the patient sample are shown in Table. Both groups were age- and sex-matched. No significant differences were found with respect to heart rate, systolic or diastolic blood pressure, duration of symptoms, frequency distribution of symptoms, New York Heart Association functional class, and cardiovascular risk factors among patients with DCM and iCMP (Table). Although a trend toward a more impaired left ventricular ejection fraction was observed for patients with DCM, this difference did not reach statistical significance. Enhanced human leukocyte antigen class II antigen expression on EMB was observed in 19% of patients with DCM and in all patients with iCMP (Table). In the iCMP group, viral genomes were detected in 35/75 patients (47%). Cardiotropic viruses included Epstein–Barr virus, parvovirus B19, and human herpesvirus 6 and 7. Elevated C-reactive protein levels were detected in 68% of patients with iCMP as compared with 40% of patients with DCM. Troponin T levels were comparable between patients with DCM and iCMP. Importantly, estimated glomerular filtration rate and distribution of patients among chronic kidney disease stages were comparable between patients with DCM and iCMP.

Myocardial Fibrosis, Inflammation, and Gal-3 mRNA Expression in Patients With DCM and iCMP

Analysis of EMBs revealed a similar degree of fibrosis in both groups; patients with DCM displayed fibrotic changes on 15% (Q1–Q3, 10%–22%) of total tissue area, as compared with 17% (Q1–Q3, 9%–28%) of total tissue area in patients with iCMP (Figure 1A). On median, patients with iCMP and DCM displayed an inflammatory cell count of 28 (Q1–Q3, 24–58) and 4 (Q1–Q3, 1–9) cells/mm², respectively (Figure 1B).

For technical reasons, it was not possible to perform reverse transcriptase polymerase chain reaction of left ventricular EMBs in 5 patients with DCM and in 10 patients with iCMP. For right ventricular EMBs, reverse transcriptase polymerase chain reaction was not possible in 4 patients with DCM and in 6 patients with iCMP. Left ventricular Gal-3 mRNA levels were comparable between patients with DCM and in patients with iCMP, whose inflammatory cell count on EMB was below median (Figure 1C). However, patients with an inflammatory cell count above median demonstrated higher left ventricular Gal-3 mRNA expression as compared with subjects with an inflammatory cell count below median (Figure 1C). Duration of symptoms in patients with iCMP was not related to left ventricular inflammation, fibrosis, or Gal-3 mRNA expression (data not shown). No differences were observed for right ventricular Gal-3 mRNA expression between patients with DCM and iCMP (Figure 1D).

Association of Endomyocardial Gal-3 Expression With Cardiac Fibrosis and Inflammation

We next assessed whether endomyocardial Gal-3 levels are associated with myocardial fibrosis in both groups of patients.

Table.   Patient Characteristics

	Nonischemic DCM (n=40)	Inflammatory Cardiomyopathy (n=75)	P Value
Age, y (SD)	46±16	44±13	0.51
Female, n (%)	12 (31)	24 (32)	0.73
BMI, kg/m² (SD)	27±7	28±5	0.82
Heart rate, beats per minute (SD)	71±9	76±14	0.30
Systolic BP, mm Hg (SD)	122±14	128±16	0.41
Diastolic BP, mm Hg (SD)	77±9	81±10	0.39
Duration of symptoms in days, median (IQR)	8 (3–105)	13 (4–31)	0.49
Symptoms, n (%)	37 (93)	71 (95)	0.77
Dyspnea	23 (58)	35 (47)	0.38
Fatigue	20 (50)	29 (39)	0.25
Peripheral edema	11 (27)	7 (9)	0.17
Chest pain	18 (46)	38 (51)	0.69
NYHA functional class, n (%)			
I	17 (42)	40 (53)	0.54
II	14 (35)	27 (36)	0.78
III	6 (15)	5 (7)	0.35
IV	3 (8)	3 (4)	0.41
LV ejection fraction, median (IQR)	28 (21–46)	42 (26–55)	0.06
Pathological ECG findings, n (%)	20 (50)	40 (53)	0.70
Elevated troponin T, n (%)	26 (65)	47 (63)	0.84
Elevated CRP, n (%)	16 (40)	51 (68)	0.04
eGFR, mL/min/1.73 m² (SD)	74±29	78±31	0.62
CKD stage, n (%)			
I	7 (18)	19 (26)	0.55
II	25 (63)	43 (57)	0.73
III	6 (15)	10 (13)	0.69
IV	2 (4)	3 (4)	0.84
Cardiovascular risk factors, n (%)	26 (65)	42 (56)	0.21
Hypertension	18 (46)	33 (44)	0.67
Smoking	11 (27)	25 (33)	0.58
Diabetes mellitus	8 (19)	7 (9)	0.19
Dyslipidemia	12 (31)	18 (24)	0.35
Obesity	15 (38)	28 (37)	0.83
Results of LV-EMB, mean (SD)			
Number of CD3-positive T-lymphocytes	3 (3)	15 (9)	<0.001
Number of CD68-positive macrophages	10 (6)	33 (24)	<0.001
Viral genome detection, n (%)	8 (19)	35 (47)	<0.01
Enhanced MHC-II antigen expression, n (%)	4 (11)	75 (100)	<0.001

BMI indicates body mass index; BP, blood pressure; bpm, beats per minute; CKD, chronic kidney disease; CRP, C-reactive protein; DCM, dilated cardiomyopathy; eGFR, estimated glomerular filtration rate; IQR, interquartile range; LV, left ventricle; LV-EMB, endomyocardial biopsy of the left ventricle; MHC, major histocompatibility complex; NYHA, New York Heart Association; and SD, standard deviation.
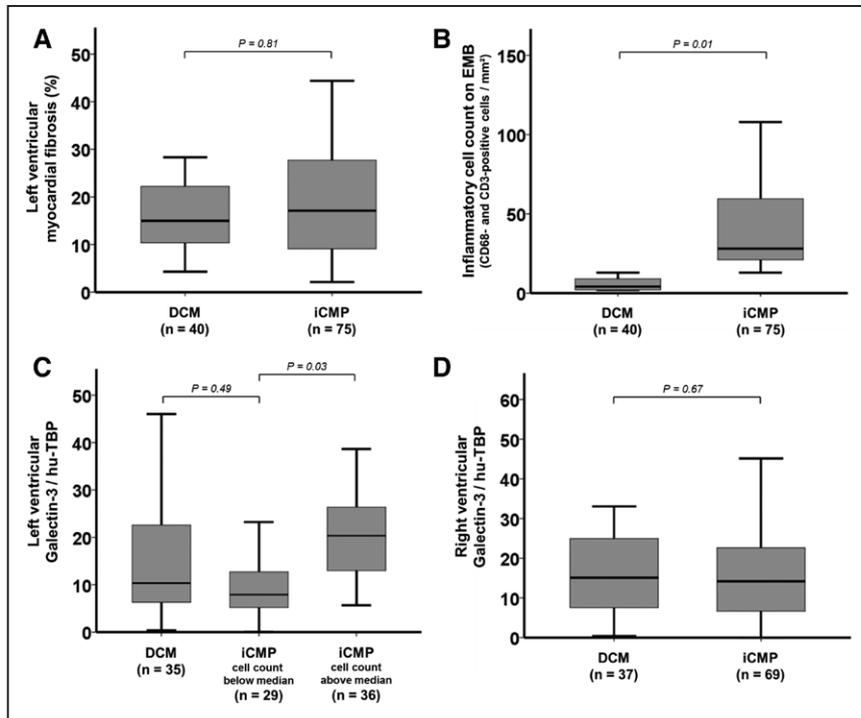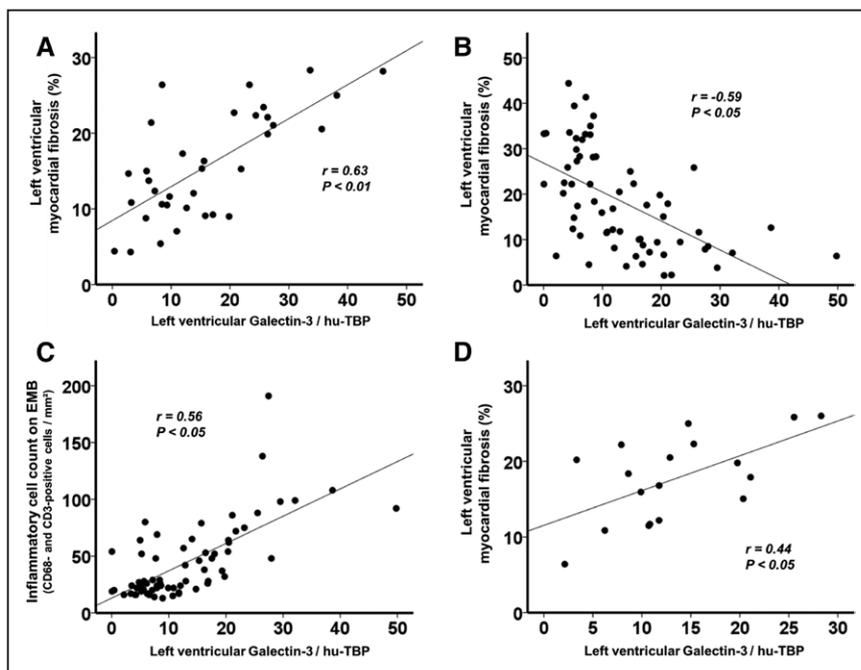
Figure 1. Quantification of myocardial fibrosis, inflammation, and galectin-3 (Gal-3) levels in patients with dilated cardiomyopathy (DCM) and inflammatory cardiomyopathy (iCMP). **A**, The degree of myocardial fibrosis was quantified in left ventricular endomyocardial biopsies stained with Masson's trichrome. **B**, Endomyocardial biopsies were stained with antibodies against CD3 and CD68 to assess myocardial inflammation. The amount of Gal-3 in endomyocardial biopsies from the left (**C**) and right ventricle (**D**) was analyzed by reverse transcriptase polymerase chain reaction (RT-PCR). EMB indicates endomyocardial biopsies; and TBP, TATA-binding protein.

In line with the current concept of Gal-3 as a marker linked to tissue fibrosis, Gal-3 mRNA expression was positively correlated with the degree of myocardial fibrosis in patients with DCM (r=0.63; P<0.01; Figure 2A). However, in patients with iCMP, a weak inverse association was observed between Gal-3 mRNA levels and fibrotic areas on EMB (r=−0.59; P<0.05; Figure 2B). Notably, in these patients, cardiac Gal-3 positively correlated with the degree of myocardial inflammation (ie, the inflammatory cell count on EMB; r=0.56; P<0.05; Figure 2C). Only in those iCMP patients with mild degree of inflammation (ie, patients in the lowest tertile grouped by inflammatory cell count), a weak, but significant positive

association of Gal-3 with myocardial fibrosis was observed (r=0.44; P<0.05; Figure 2D).

Gal-3 Protein Expression and Fibrosis in Patients With DCM and iCMP

We further investigated protein expression of Gal-3 in EMBs from patients with DCM and iCMP by immunohistochemistry. No relevant background fluorescence was observed on EMB slides (Figure I in the Data Supplement). Consistent with findings obtained for mRNA expression, patients with DCM and mild fibrosis on EMB displayed low Gal-3 staining, whereas those with enhanced fibrosis exhibited stronger Gal-3

Figure 2. Spearman correlation analysis for the association of endomyocardial galectin-3 (Gal-3) levels with (**A**) myocardial fibrosis in patients with dilated cardiomyopathy (DCM; n=35), (**B**) myocardial fibrosis in patients with inflammatory cardiomyopathy (iCMP; n=67), (**C**) inflammatory cell count in patients with iCMP (n=72), and (**D**) myocardial fibrosis in patients with iCMP and inflammatory cell count in the lower tertile (n=18). EMB indicates endomyocardial biopsies; and TBP, TATA-binding protein.

expression (Figure 3A). In contrast, for patients with iCMP and comparable amounts of myocardial fibrosis, those with enhanced inflammation showed more profound Gal-3 expression than patients with mild inflammation (Figure 3B).

Lack of Correlation Between Plasma Gal-3 Levels and Endomyocardial Gal-3 Levels or Myocardial Fibrosis

To analyze whether circulating levels of Gal-3 can reflect endomyocardial Gal-3 levels, plasma levels of Gal-3 were determined by ELISA in both groups of patients. Plasma levels of Gal-3 did not differ between patients with DCM and iCMP (Figure 4A). No relevant correlation was observed between plasma levels of Gal-3 and endomyocardial Gal-3 mRNA expression for patients with DCM ($r=-0.11$; $P=0.65$; Figure 4B) and iCMP ($r=0.14$; $P=0.58$; Figure 4C). Notably, plasma levels of Gal-3 also did not correlate with left ventricular fibrosis, neither in patients with DCM ($r=-0.21$; $P=0.33$; blue asterisks; Figure 4D) nor in those with iCMP ($r=0.05$; $P=0.41$; green dots; Figure 4D). When circulating Gal-3 levels and the inflammatory cell count on EMB were compared in patients with iCMP, a weak positive correlation was observed ($r=0.25$; $P<0.05$; Figure 4E).

Discussion

The present study demonstrates that endomyocardial Gal-3 levels are associated with the histologically proven extent of fibrosis on EMB in patients with nonischemic DCM, thereby adding to the concept of myocardial Gal-3 as an estimate for cardiac fibrosis. However, this seems only to be accurate in the absence of myocardial inflammation because patients with iCMP display an inverse correlation between endomyocardial Gal-3 levels and fibrosis. Indeed, in these patients, endomyocardial Gal-3 levels rather correlate with the extent of myocardial inflammation, as assessed by the inflammatory cell count on EMBs. At least in the present cohort of patients with

DCM and iCMP, no associations between circulating levels of Gal-3 and endomyocardial Gal-3 levels or cardiac fibrosis were observed.

To the best of our knowledge, this is the first study investigating the association of circulating and biventricular myocardial Gal-3 expression with myocardial fibrosis and inflammation. The inclusion of patients with DCM versus those with iCMP allowed us to assess the link between plasma/endomyocardial Gal-3 levels and interstitial fibrosis without the confounding effect of inflammation, on the one hand, and to link Gal-3 levels with pathology when fibrosis and inflammation coexist. Of note, New York Heart Association functional class distribution and renal function, an important determinant of circulating Gal-3 levels, were similar between both groups of patients.

Myocardial fibrosis is associated with nearly all forms of heart disease and may cause adverse cardiac remodeling, functional decline, and even failure.[1,21] However, noninvasive monitoring of cardiac fibrosis remains a major challenge in the clinical setting. Although histological assessment of EMBs, as performed in the present study, is considered as the most accurate method to quantify cardiac fibrosis,[22] all patients who undergo EMB are at small but existing risk of complications. With the advent of new imaging modalities, such as cardiac magnetic resonance imaging, postcontrast T1 mapping, and estimation of the extracellular volume fraction, a noninvasive alternative has emerged.[23] Yet, standardization techniques for quantitative analysis of fibrosis and validation of these imaging techniques in larger cohorts of patients, especially in the presence of inflammation, are still needed. Therefore, in recent years, interest has increased in finding circulating biomarkers that may reflect adverse cardiac remodeling and fibrosis.

Initial observations from experimental studies led to the hypothesis that Gal-3 may serve as a novel promising marker for cardiac fibrosis.[3] Although this hypothesis has never been tested in detail in human studies, recent clinical studies have
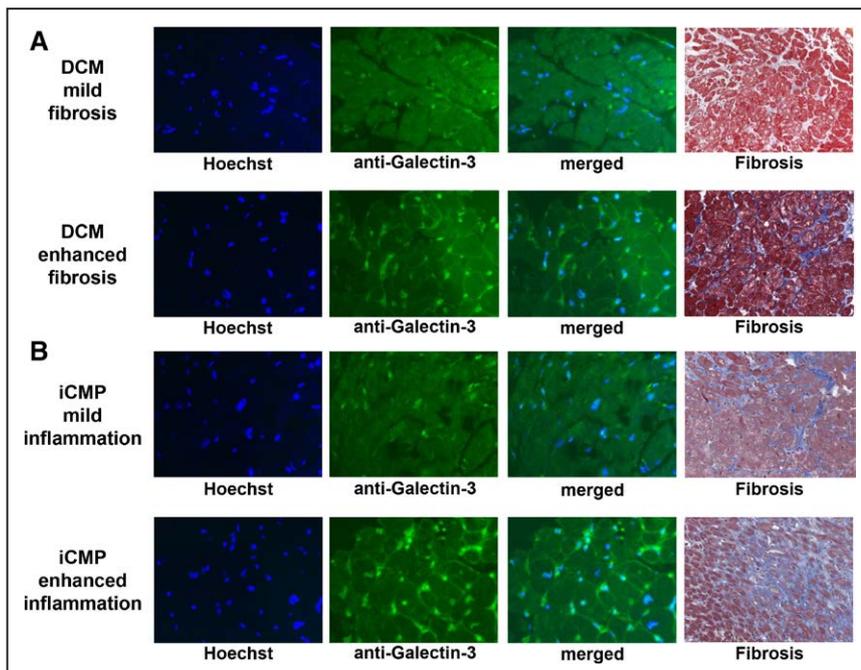
Figure 3. Immunohistochemical analysis of galectin-3 (Gal-3) protein expression in endomyocardial biopsies from patients with (**A**) dilated cardiomyopathy (DCM) and mild or enhanced fibrosis, as detected by Masson's trichrome staining, and (**B**) patients with inflammatory cardiomyopathy (iCMP) and mild or enhanced myocardial inflammation.
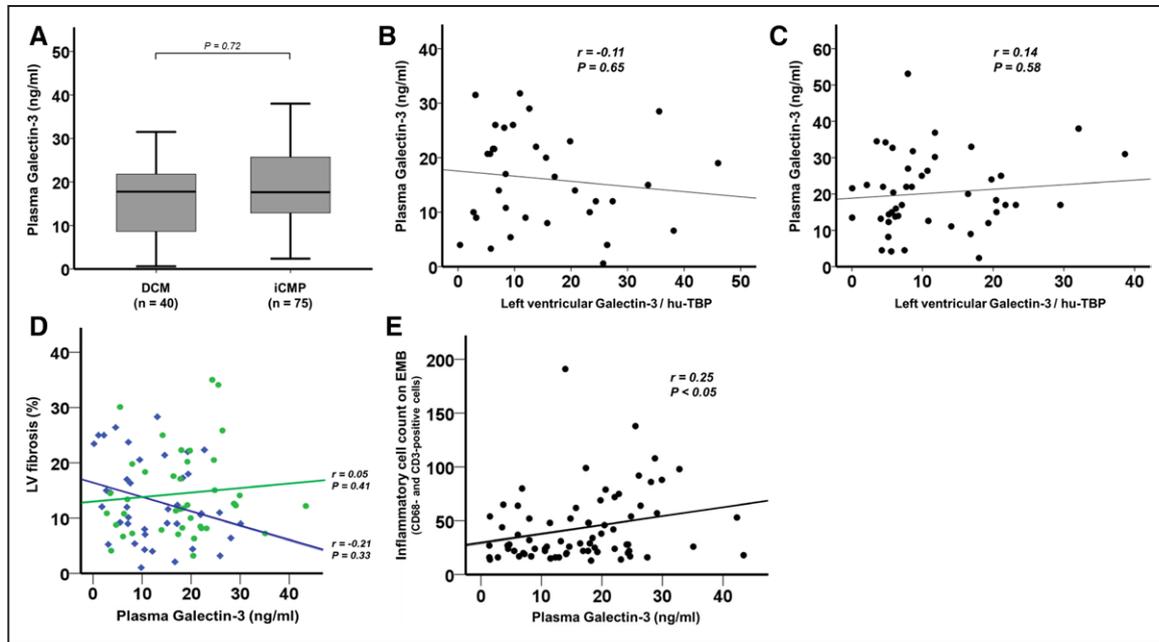
Figure 4. Association between endomyocardial and plasma levels of galectin-3 (Gal-3). **A**, Plasma Gal-3 levels were analyzed by ELISA in patients with dilated cardiomyopathy (DCM) and inflammatory cardiomyopathy (iCMP). **B**, Spearman correlation analysis for the association of Gal-3 levels in plasma and endomyocardial biopsy in patients with DCM (n=33) and (**C**) iCMP (n=45). **D**, Association between plasma concentration of Gal-3 and myocardial fibrosis as assessed by histology in patients with DCM (blue asterisks) and iCMP (green dots). **E**, Correlation analysis between plasma concentration of Gal-3 and myocardial inflammation as assessed by inflammatory cell count on endomyocardial biopsy in patients with iCMP. EMB indicates endomyocardial biopsies; LV, left ventricle; and TBP, TATA-binding protein.

investigated the prognostic value of plasma levels of Gal-3 in the community setting, as well as in a variety of patient populations with heart failure. Higher plasma concentrations of Gal-3 were associated with all-cause mortality and risk of heart failure in the general population.[24,25] Furthermore, increased plasma levels of Gal-3 were associated with adverse outcome in patients with acute[26] and chronic heart failure with reduced or preserved ejection fraction.[27–29]

The present study provides histological evidence that endomyocardial but not circulating levels of Gal-3 are associated with the extent of myocardial fibrosis in patients with noninflammatory DCM. Although the exact mechanisms mediating these effects in humans warrant further investigation, the present study supports the hypothesis that cardiac Gal-3 plays a role in the development of myocardial fibrosis. However, as also suggested by López et al,[12] Gal-3 has been implicated in a variety of biological processes, including cell adhesion, apoptosis, and inflammation. In addition, myocardial Gal-3 levels may vary in patients with heart failure, depending on the pathogenesis and stage of disease. This may, at least in part, serve as an explanation for the lack of association between plasma and myocardial Gal-3 levels, as well as between plasma Gal-3 levels and cardiac fibrosis in the present study, and the moderate correlation between plasma and myocardial Gal-3 protein levels in patients with chronic stable heart failure of hypertensive origin, as observed by López et al.[12]

In contrast to patients with noninflammatory DCM, endomyocardial Gal-3 levels in patients with iCMP were not associated with cardiac fibrosis, but rather with myocardial inflammation as detected by the inflammatory cell count on

EMB. In patients with iCMP and an inflammatory cell count in the lower tertile, endomyocardial Gal-3 levels still correlated with myocardial fibrosis. However, with increasing myocardial inflammation, Gal-3 is rather associated with the inflammatory cell count and, therefore, reflects myocardial inflammation. These findings are in line with the pleiotropic biological functions of Gal-3 and extend the findings of a recent study in patients with other noncardiac inflammatory conditions, such as pneumonia and sepsis, who displayed elevated plasma levels of Gal-3.[30] Considering the correlation between myocardial Gal-3 and the inflammatory cell count in the present study, one can hypothesize that Gal-3 release is linked to inflammatory cells. A recent study, however, suggested that macrophages are not the only source of Gal-3 in the myocardium in a mouse model of transverse aortic constriction.[31] Early upregulation of Gal-3 after pressure overload was localized in subpopulations of macrophages and myofibroblasts. Of note, after 1 to 4 weeks of transverse aortic constriction, a subset of cardiomyocytes in fibrotic areas contained large amounts of Gal-3.[31] Further studies are needed to clarify whether inflammatory cells are indeed the source of myocardial Gal-3 in iCMP and which type of cell is responsible for it.

The finding of myocardial Gal-3 as a marker for both cardiac inflammation and fibrosis is of importance because fibrosis and inflammation have to be assumed to be coincident in a relevant number of patients with symptoms of heart failure.[9] Given that various degrees of myocardial fibrosis and inflammation can be present in a patient, Gal-3 levels may reflect a sum of these different pathologies, but will no longer inform solely on the extent of myocardial fibrosis. Consequently,

these observations justify further research on the role of myocardial Gal-3 as a potential modifier of myocardial fibrosis and inflammation, but highlight the fact that Gal-3 is not suitable to estimate the one or the other independently.

The interest in Gal-3 arises from the potential to serve as a biomarker. As such, not myocardial but circulating Gal-3 got into the focus of recent investigations. However, the present data could not demonstrate that plasma levels of Gal-3 are helpful in reflecting endomyocardial Gal-3 levels or myocardial pathologies, neither fibrosis nor inflammation. This is in keeping with 2 reports in patients with end-stage heart failure[10,11] and the study by López et al,[12] the latter demonstrating a weak correlation with overt scatter.

These findings might not be surprising given the nearly ubiquitous expression of Gal-3 in several organs,[8] which likely contribute to plasma levels of Gal-3. Notably, Calvier et al[32] recently demonstrated that Gal-3 expression is upregulated in vascular smooth muscle cells on stimulation with aldosterone and mediates aldosterone-induced vascular fibrosis in vivo, suggesting that the vasculature may provide another potential source of circulating Gal-3 in patients with cardiovascular pathologies.

Although circulating Gal-3 levels seem not to inform reliably on myocardial Gal-3 levels, it should be acknowledged that circulating Gal-3 have been shown as predictor for outcome in several cohort studies, as mentioned earlier.[24–29] Assuming that Gal-3 is linked to various pathological processes, including fibrosis and inflammation, increases in Gal-3 are likely to indicate worse outcome, but does not allow to inform on pathology, mechanisms, or contributing components of disease. Importantly, results from clinical studies evaluating the prognostic value of plasma Gal-3 are not entirely consistent. Plasma levels of Gal-3 failed to improve risk stratification in a larger study of patients with heart failure with reduced ejection fraction after multivariate analysis.[33] In another study, no increase in plasma Gal-3 levels was observed in patients with heart failure with reduced ejection fraction who died or underwent heart transplantation as compared with those without event.[34] Although some studies indicated that plasma Gal-3 is a predictor of adverse outcome in heart failure independent of proBNP (pro-B-type natriuretic peptide) level, others demonstrated a lack of prognostic benefit of Gal-3 after adjusting for proBNP levels[35] or renal function.[36] Several reasons may account for the contradictive results on the predictive value of Gal-3 in these studies, such as heterogeneities in the patient populations studied or methodological difference among the studies. Nevertheless, these findings suggest that apart from a potential role as outcome predictor, additional pathophysiological insight is needed on the regulation of Gal-3 levels in patients with heart failure.

Interestingly, the present data suggest an inverse relationship between endomyocardial levels of Gal-3 and left ventricular fibrosis. The reason for this inverse association remains unclear, but may relate to the fact that patients with iCMP presented in the subacute to chronic phase of iCMP, where Gal-3 levels are probably determined by inflammation, fibrosis, and other, not yet defined, biological processes. Although a higher inflammatory cell count in patients with less fibrosis

on EMB could serve as an explanation for the inverse relationship mentioned earlier, this difference did not reach statistical significance in patients with iCMP in the present study (data no shown). Future experimental studies should, therefore, aim to characterize the time-dependent relationship between myocardial inflammation, fibrosis, and circulating/myocardial Gal-3 levels in more detail by analyzing serial sets of blood samples and EMB.

A potential limitation of the present study is that protein expression of Gal-3 in EMBs was analyzed by immunohistochemistry. However, because of the limited amount of biopsy material, it was not possible to apply a more rigorous method of quantification, such as immunoblotting or ELISA. Immunohistochemical analysis of Gal-3 in biopsy specimens from patients with DCM and iCMP displayed a diffuse interstitial staining pattern, rather than focal distribution. Nevertheless, a potential sampling error needs to be considered in the interpretation of endomyocardial Gal-3 levels in the present study. Recent observations suggest that markers of collagen metabolism, such as N-terminal propeptide of procollagen type III (PIIINP) or C-terminal propeptide of procollagen type I (PICP), are likely better biomarkers of myocardial fibrosis, and circulating levels of these proteins have been validated against histopathologic analysis of fibrosis on EMB.[22] However, these markers were not tested in the present study.

In conclusion, the present study demonstrates for the first time that myocardial Gal-3 needs to be considered as a marker for both cardiac inflammation and fibrosis. At least in the present cohort, plasma Gal-3 did not serve as a reliable estimate for endomyocardial Gal-3 levels. These findings are likely of relevance for studies evaluating plasma Gal-3 as a novel biomarker in patients with heart failure and highlight the importance to strictly validate whether a circulating biomarker indeed reflects histologically proven fibrosis. Finally, contradicting findings in the field of Gal-3 may be explained by difficulties in comparing studies in terms of methodology, patient populations, and varying etiologies or stages of disease, but also underscore the complexity of Gal-3 regulation and interactions in cardiovascular disease.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Cardiac fibrosis is a key pathological feature of nearly all forms of heart disease. Circulating levels of galectin-3 are currently intensely investigated as a potential novel biomarker for cardiac fibrosis in heart failure. However, validation that galectin-3 indeed reflects histologically proven myocardial fibrosis in patients with newly diagnosed cardiomyopathy is lacking. The findings of the present study suggest that myocardial galectin-3 needs to be considered as marker for both inflammation and fibrosis. Given that various degrees of myocardial fibrosis and inflammation can be present in a patient, galectin-3 levels may reflect a sum of these different pathologies, but will no longer inform solely on the extent of myocardial fibrosis. Furthermore, when tested on an individual basis, circulating galectin-3 levels neither reflect myocardial galectin-3 levels nor histologically proven myocardial fibrosis, highlighting the importance to more strictly validate whether a given circulating biomarker indeed serves as an indicator of a biological or pathological process before it is applied clinically.

Plasma and Cardiac Galectin-3 in Patients With Heart Failure Reflects Both Inflammation and Fibrosis: Implications for Its Use as a Biomarker

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SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Background fluorescence observed during immunohistochemical detection of Gal-3 in endomyocardial biopsies.
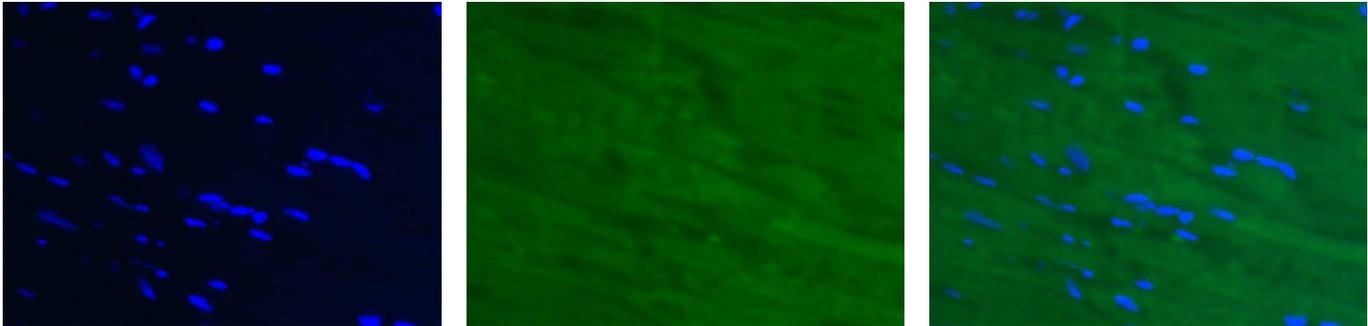
Hoechst   anti-Galectin-3   merged