

# Relationships between circulating galectin-3, extracellular matrix fibrosis and outcomes in dilated cardiomyopathy

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## Conflict of interest

None declared

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## Abstract

**Background.** Galectin-3 is an emerging biomarker in cardiovascular disease. Myocardial galectin-3 is involved in the pathology of cardiac fibrosis; however, the role of circulating galectin-3 is not yet established.

**Objectives.** To assess the relationships between circulating galectin-3, fibrosis and outcomes in dilated cardiomyopathy (DCM).

**Materials and methods.** We included 70 patients (age:  $48 \pm 12.1$  years, ejection fraction (EF)  $24.4 \pm 7.4\%$ ) with new-onset DCM ( $n = 35$ ,  $\leq 6$  months). Galectin-3 and procollagen type I and III (PICP, PINP, PIIICP, and PIIINP), transforming growth factor  $\beta$  (TGF- $\beta$ ), connective tissue growth factor (CTGF), osteopontin (OPN), matrix metalloproteinases (MMP-2 and -9), and tissue inhibitor (TIMP-1) were determined in serum at baseline and after 3 and 12 months. Patients underwent endomyocardial biopsy. The endpoint was a combination of death and urgent hospitalization at 12 months.

**Results.** Galectin-3 did not correlate with biopsy-determined fibrosis. Baseline galectin-3 correlated with OPN, TIMP-1, PIIICP, and MMP-2. In new-onset DCM, galectin-3 levels at baseline were higher than at 3 and 12 months, whereas in chronic DCM there was no difference. Galectin-3 was a predictor of the endpoint (hazard ratio (HR) = 1.115; 95% confidence interval (95% CI) = 1.009–1.231;  $p < 0.05$ ). The best cut-off value was 14.54 ng/mL (area under the curve (AUC) = 0.67). Patients with galectin-3  $\geq 14.54$  ng/mL had an increased risk of events (HR = 2.569; 95% CI = 1.098–6.009;  $p < 0.05$ ).

**Conclusions.** Circulating galectin-3 is unrelated to fibrosis. Serial measurements of galectin-3 correlated with markers of fibrosis, including markers of collagen synthesis and OPN. Circulating galectin-3 was independently associated with cardiovascular (CV) outcomes in DCM.

**Key words:** fibrosis, kinetics, galectin-3, dilated cardiomyopathy

## Background

Fibrosis of the extracellular matrix (ECM) is a common pathway in many cardiac diseases leading ultimately to the development of heart failure (HF).<sup>1</sup> Patients with dilated cardiomyopathy (DCM) are particularly predisposed to extensive ECM fibrosis, specifically reactive fibrosis, which contributes to functional impairment and subsequent ventricular arrhythmias.<sup>2</sup> The mechanisms responsible for ECM fibrosis are incompletely described.

Galectin-3 belongs to the  $\beta$ -galactoside-binding lectin family and serves as a matricellular protein which binds basic components of ECM, such as collagens, elastin and fibronectin.<sup>3</sup> In vitro studies have revealed that galectin-3 is a pivotal protein involved in the development of ECM fibrosis, as it stimulates transdifferentiation of fibroblasts into highly active myofibroblasts that produce ECM compounds, e.g., collagens and elastin, in excess.<sup>4</sup> Additionally, galectin-3 is an important component of the renin–angiotensin–aldosterone pathway that enhances ECM fibrosis.<sup>5</sup> Thus, the significance of myocardial (in situ) galectin-3 in ECM fibrosis has been unequivocally proven. However, the role of circulating galectin-3 is unclear.

Numerous claims were published that circulating galectin-3 is a marker of cardiac fibrosis.<sup>6–8</sup> Upon our review of the medical literature, it seems that these assertions are premature. In fact, very few studies performed an in-depth evaluation of the associations between circulating galectin-3 and ECM fibrosis in HF.<sup>9–11</sup> In those studies, cardiac fibrosis was assessed, either invasively (by means of endomyocardial biopsy (EMB) followed by a detailed laboratory assessment of samples) or non-invasively (with magnetic resonance imaging (MRI)). The determination of late gadolinium enhancement (LGE) or post-LGE T1 relaxation time served as surrogates of ECM fibrosis. However, even in those studies, an association between circulating galectin-3 and ECM fibrosis (assessed with either method) was not clearly confirmed.

## Objectives

Although a considerable amount of work has been published on the subject of galectin-3 in cardiomyopathy and HF, we have identified knowledge gaps that warrant further exploration. The relationship between circulating galectin-3 and cardiac fibrosis is inconclusive. Thus, our study rigorously explores the associations between galectin-3 and invasively determined ECM fibrosis (expressed qualitatively and quantitatively) in a homogenous group of DCM patients. Furthermore, the relationship between galectin-3 and other serum markers of fibrosis (such as markers of collagen synthesis, fibrosis controlling factors or metalloproteinases (MMPs)) is generally unknown. The kinetics of serum galectin-3 in DCM patients stratified according to disease duration and fibrosis status

have not been well described. Finally, we aim to explore the association between galectin-3 and survival in HF in DCM patients, as few studies have reported on this relationship.

## Materials and methods

### Study population

Between July 2014 and October 2015, we enrolled 70 consecutive DCM patients who fulfilled pre-specified criteria and were willing to participate in the study. Dilated cardiomyopathy was diagnosed according to the definition of the European Society of Cardiology 2007 guidelines, after an exclusion of significant coronary artery disease (CAD), primary heart valve disease, congenital heart disease, and arterial hypertension.<sup>12</sup> Based on the duration of symptoms, patients were divided into equal groups consisting of 35 subjects with new-onset (group 1,  $\leq 6$  months) and chronic (group 2,  $> 6$  months) DCM. The duration of HF symptoms was defined as the time from the onset of subjective symptoms (dyspnea on exertion or at rest, paroxysmal nocturnal dyspnea, orthopnea, palpitations) and/or edema to the index hospitalization or ambulatory visit in cardiology clinic. Patients with the presence of concomitant non-cardiac diseases, such as bone and joint diseases, chronic liver insufficiency, peripheral atherosclerosis, and neoplasms affecting collagen metabolism and circulating levels of procollagens, were excluded. Each patient's clinical status was re-evaluated, with echocardiography and blood sampling repeated at 3 and 12 months (patients' flowchart is presented in Fig. 1). We also evaluated 20 healthy volunteers as a control group who underwent blood sampling and echocardiography. The study protocol was approved by the John Paul II Institutional Review Board and the Kraków Medical Chamber Ethics Committee (reference No. 134/KBL/OIL/2013). All patients gave a written informed consent prior to inclusion in the study.

### Endomyocardial biopsy

Endomyocardial biopsy (EMB) procedures were performed by experienced operators via a femoral or jugular vein approach. Long (104 cm), flexible, disposable 7 French biopsy forceps with small jaws (Cordis®; Johnson & Johnson Co Inc., Miami Lakes, USA) were used.<sup>13</sup> Up to 5 myocardial samples were obtained, which were immediately stored in formalin for light microscopic examination. The presence of fibrosis was determined qualitatively and quantitatively by an experienced pathologist blinded to the clinical data. Specimens for fibrosis assessment were stained with Masson's trichrome; fibrotic areas stained blue, and normal muscle fibers stained red. Collagen volume fraction (CVF) was assessed with quantitative morphometry as previously described.<sup>14</sup>

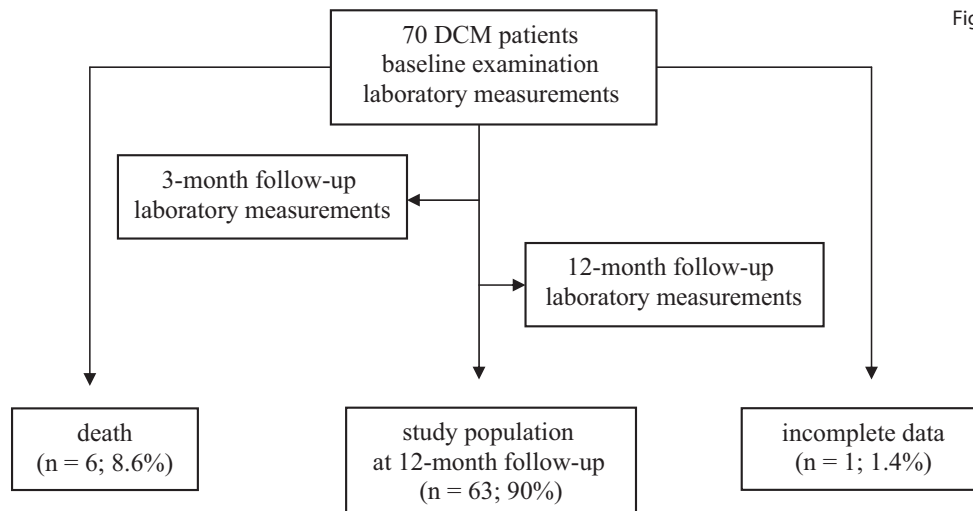


Fig. 1. Study flowchart

## Laboratory measurements

Venous blood samples for biomarkers measurements were drawn after an overnight fast, typically between 8 a.m. and 9 a.m. After centrifugation, the supernatant was stored at  $-20^{\circ}\text{C}$ . The concentration of collagen synthesis markers (carboxy- and amino-terminal propeptides of procollagen type I and III (PICP, PINP, PIIICP, and PIIINP)) and fibrosis controlling factors (transforming growth factor  $\beta$  (TGF- $\beta$ ) and connective tissue growth factor (CTGF), osteopontin (OPN), as well as matrix metalloproteinases (MMP-2 and -9) and tissue inhibitor (TIMP-1)) were determined in plasma using a commercially available enzyme-linked immunosorbent assay (ELISA), as previously described.<sup>15</sup> The levels of galectin-3 were measured with ELISA using a commercially available kit (Human Galectin-3 ELISA, RAF015R; BioVendor, Brno, Czech Republic). The serum samples and galectin-3 standard dilutions were added to microwells that were coated with anti-galectin-3 antibody. Galectin-3 present in the sample or standard was bound to antibodies adsorbed to the microwells. Following incubation, a wash step was performed and the horseradish peroxidase (HRP)-conjugated anti-galectin-3 antibodies were added binding to galectin-3 captured by the first antibody. Again following incubation, unbound HRP-conjugated anti-galectin-3 antibodies were removed during a wash step, and a substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of galectin-3 present in the sample or standard. The reaction was terminated by the addition of acid and absorbance was measured at 450 nm. The galectin-3 sample concentration was determined based on the standard curve. Baseline, 3- and 12-month measurements of galectin-3, PICP, PINP, PIIICP, PIIINP, TGF- $\beta$ , CTGF, and OPN were obtained; however, for MMP-2, MMP-9 and TIMP-1, only baseline measurements were available. Intra-assay and inter-assay coefficients of variation were  $<7\%$ .

## Statistical analysis

The data are presented either as mean  $\pm$  standard deviation (SD), median and interquartile range (IQR), or count and percentages. The normality of the distribution of variables was assessed with a Shapiro–Wilk test. Comparisons of clinical parameters between 2 groups were conducted with a Mann–Whitney U test, as a lack of normality was found. Univariate relationships between galectin-3 and serum markers of fibrosis were determined with Spearman correlation analysis. Two endpoints were analyzed: cardiovascular (CV) death and the combined endpoint that was composed of CV death and urgent HF hospitalization at 12 months. Survival data were analyzed using the Kaplan–Meier method, and compared with the log-rank test. To examine the associations of galectin-3 with endpoints of interest (unadjusted analyses and analyses adjusted for age, duration of disease, CVE, ejection fraction (EF), and N-terminal pro-B-type natriuretic peptide (NT-proBNP)) Cox proportional hazard analyses were performed. Calculations for the optimal cut-off values of galectin-3 (in order to determine the cut-off values for adverse outcomes) were carried out using a receiver operating characteristic (ROC) curve. Patients were compared according to a galectin-3 optimal cut-off value, derived from ROC analysis, with the use of a log-rank test. All results were considered statistically significant when the p-value was  $<0.05$ . All the analyses were conducted in R software v. 3.3.2 (The R Foundation for Statistical Computing, Vienna, Austria).

## Results

### Baseline characteristics

Table 1 shows the baseline characteristics of the study population. The majority of patients were male (63; 90%) with symptomatic HF (New York Heart Association (NYHA)

**Table 1.** Baseline characteristics of the study population

Parameter	DCM (n = 70)
Age [years]	48 ±12.1
Sex [male/female]	63 (90%)/7 (10%)
BMI [kg/m <sup>2</sup> ]	26.8 ±5.4
NYHA class	2.49 ±0.7
Duration [months]	24.3 ±35.6
LBBB [n, %]	18 (25.7%)
LVESd/BSA [mm/m <sup>2</sup> ]	30.1 ±7.1
LVEDd/BSA [mm/m <sup>2</sup> ]	35.6 ±7.0
LVESvol/BSA [mL/m <sup>2</sup> ]	96.1 ±49.0
LVEDvol/BSA [mL/m <sup>2</sup> ]	126.8 ±59.8
EF [%]	24.4 ±7.4
E/E' (average sep+years)	20.8 ±11.4
ECM fibrosis [n, %]	24 (34.3%)
PA mean [mm Hg]	23.1 ±10.9
PH [n, %]	27 (39.7%)
VO <sub>2</sub> peak [mL/kg/min]	16.5 ±6.1
Hb [g/dL]	14 ±1.6
hs-troponin T [ng/mL]	0.022 ±0.018
hs-CRP [mg/dL]	9.52 ±23.6
NT-proBNP [pg/mL]	3373 ±5428
β-blocker [n, %]	69 (98.6%)
ACE-I or ARB [n, %]	68 (97.1%)
MRA [n, %]	66 (94%)
Furosemide [n, %]	42 (60%)
CRT-D [n, %]	20 (28.6%)

Data are presented as mean ±SD or n (%); DCM – dilated cardiomyopathy; BMI – body mass index; NYHA – New York Heart Association; LBBB – left bundle branch block; LVESd – left ventricle end-systolic diameter indexed to body surface area; LVEDd – left ventricle end-diastolic diameter indexed to body surface area; EF – ejection fraction; E/E' – ratio of early mitral inflow velocity to early mitral myocardial velocity; ECM – extracellular matrix; PA mean – mean pulmonary pressure; VO<sub>2</sub> peak – peak oxygen uptake; Hb – hemoglobin; hs-CRP – high-sensitivity C-reactive protein; NT-proBNP – N-terminal pro-B-type natriuretic peptide; ACE-I – angiotensin-converting enzyme inhibitors; ARB – angiotensin II receptor blockers; MRA – mineralocorticoid receptor antagonists; CRT-D – cardiac resynchronization therapy with cardioverter/defibrillator.

class 2.49 ±0.7). All patients had severely remodeled left ventricle (LV; indexed to BSA left ventricle end-systolic (LVES) volume 96.1 ±49 mL/m<sup>2</sup> and left ventricle end-diastolic (LVED) volume 126.8 ±59.8 mL/m<sup>2</sup>) with significantly depressed LV systolic (EF 24.4 ±7.4%) and diastolic

(E/E' 20.8 ±11.4) function. Approximately 1/3 (24 (34.3%)) of patients had ECM fibrosis diagnosed with EMB. Patients had significantly increased serum NT-proBNP level (3373 ±5428 pg/mL). All patients were on optimal medical therapy: β-blockers in 69 (98.6%), angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers (ACE-I/ARB) in 68 (97.1%), mineralocorticoid receptor antagonists (MRA) in 66 (94%), and implantable cardiac device with or without cardiac resynchronization therapy (ICD ±CRT) in 20 (28.6%).

### Comparison of baseline, 3- and 12-month galectin-3 between DCM patients and control group

The control group consisted of 20 healthy subjects that were previously characterized.<sup>15</sup> Comparison of the baseline, 3- and 12-month serum galectin-3 values of DCM patients with baseline values of 20 control subjects is presented in Table 2. All measurements were significantly higher in DCM patients compared to controls.

### Relationships between galectin-3, invasively determined ECM fibrosis and serum markers of fibrosis

Baseline galectin-3 did not correlate with either qualitative ECM fibrosis assessment ( $r = -0.13$ ,  $p = 0.29$ ) or quantitative measurement, expressed as CVF ( $r = -0.12$ ,  $p = 0.58$ ). Baseline galectin-3 correlated with the following baseline markers of fibrosis: OPN (0.27;  $p < 0.02$ ), TIMP-1 (0.23;  $p < 0.03$ ), PIICP (0.27;  $p < 0.03$ ), and MMP-2 (0.27;  $p < 0.03$ ). Galectin-3 at 3-month follow-up correlated with the following 3-month markers: OPN (0.37;  $p < 0.004$ ), PINP (0.27;  $p < 0.03$ ) and PIINP (0.34;  $p < 0.006$ ). Galectin-3 at 12-month follow-up correlated only with 12-month OPN (0.35;  $p < 0.005$ ) and PIICP (0.29;  $p < 0.04$ ).

### Kinetics of galectin-3 in new-onset and chronic DCM

In patients with new-onset DCM, serum levels of galectin-3 at baseline were significantly higher than galectin-3 levels at 3- and 12-month follow-up (14.01 ng/mL (11.17–17.8 ng/mL) compared to 12.42 ng/mL (10.34–14.59 ng/mL) compared to 12.32 ng/mL (10.22–15.26 ng/mL), respectively;

**Table 2.** Comparison of serum galectin-3 between DCM patients and control group at baseline, 3 and 12-month follow-up

Parameter	DCM (n = 70)	Control (n = 20)	p-value
Baseline galectin-3 [ng/mL]	14.26 (11.03–17.47)	9.84 (8.6–10.9)	<0.001
3-month galectin-3 [ng/mL]	12.5 (10.22–15.07)	9.84 (8.6–10.9)	0.002
12-month galectin-3 [ng/mL]	13.23 (10.43–16.06)	9.84 (8.6–10.9)	<0.001

Data are presented as median and interquartile range; DCM – dilated cardiomyopathy.

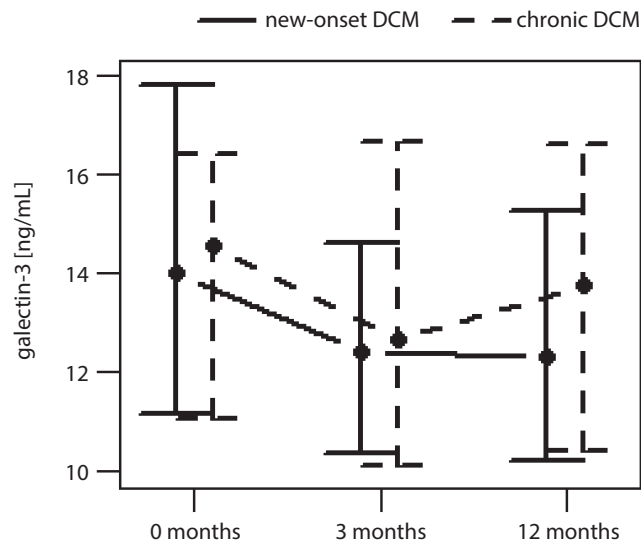


Fig. 2. 12-month patterns of serum galectin-3 in new-onset and chronic DCM

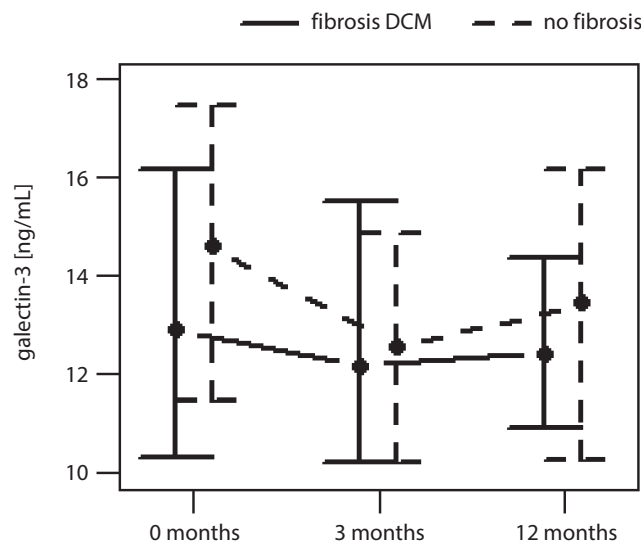


Fig. 3. 12-month patterns of serum galectin-3 in DCM with and without fibrosis

$p < 0.001$ ). Of note, there was no difference between values at 3- and 12-month follow-up. In contrast, galectin-3 levels in patients with chronic DCM were similar at baseline and at 3 and 12 months (14.54 ng/mL (11.04–16.39 ng/mL) compared to 12.66 ng/mL (10.12–16.65 ng/mL) compared to 13.74 ng/mL (10.43–16.59 ng/mL), respectively;  $p = 0.18$  and  $p = 0.58$ , respectively). Similarly, there were no differences between 3- and 12-month follow-up ( $p = 0.27$ ). The kinetics of galectin-3 over the 12-month follow-up are presented separately for new-onset and chronic DCM groups in Fig. 2 and Fig. 3. The kinetics of galectin-3 in new-onset DCM are characterized by a decreasing pattern (Fig. 2); in chronic DCM, the kinetics of galectin-3 are flat. Comparisons of baseline, 3- and 12-month galectin-3 values between new-onset and chronic DCM revealed that there were no differences between the 2 groups (Table 3, Fig. 4).

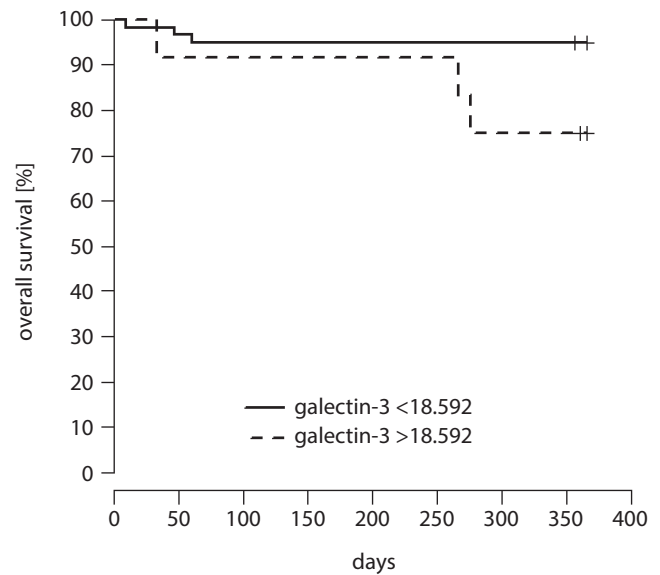


Fig. 4. Kaplan-Meier 12-month survival curves for galectin-3 cut-off value of 18.592 ng/mL

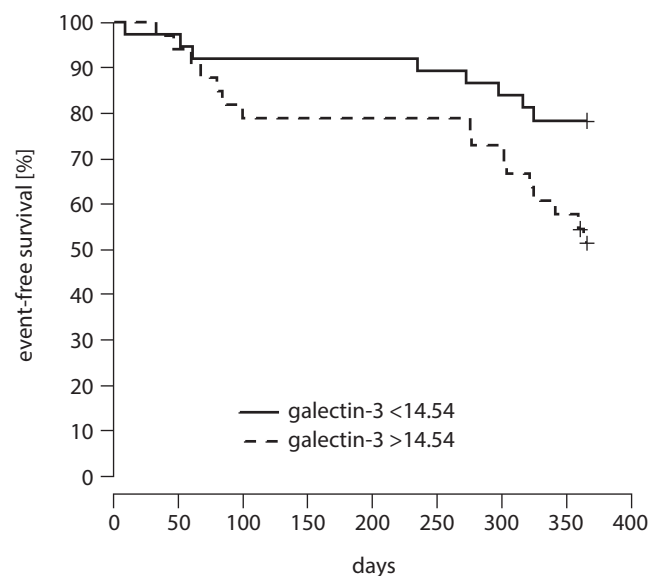


Fig. 5. Kaplan-Meier 12-month event-free curves for galectin-3 cut-off value of 14.54 ng/mL

## Kinetics of galectin-3 in DCM patients with and without fibrosis

Galectin-3 serum levels were similar at the index visit, 3- and 12-month follow-up in patients with ECM fibrosis (12.9 ng/mL (10.33–16.18 ng/mL) compared to 12.8 ng/mL (10.23–15.49 ng/mL) compared to 12.43 ng/mL (10.89–14.38 ng/mL);  $p = 0.64$  and  $p = 0.99$ , respectively). No differences were observed between 3- and 12-month measurements ( $p = 0.54$ ). However, in patients without ECM fibrosis, galectin-3 levels significantly decreased between baseline and 3-month follow-up (14.61 ng/mL (11.46–17.47 ng/mL) compared to 12.58 ng/mL (10.23–14.87 ng/mL);  $p < 0.001$ ). Although galectin-3 levels



**Table 3.** Correlations between galectin-3 and serum markers of fibrosis, measured at baseline and at 3- and 12-months follow-up

Parameter	Baseline measurements r; p-value	3-month measurements r; p-value	12-month measurements r; p-value
PICP [ng/mL]	−0.08; 0.52	0.01; 0.92	0.07; 0.63
PINP [pg/mL]	−0.06; 0.64	0.27; 0.03	0.17; 0.25
PIIICP [ng/mL]	0.27; 0.03	0.19; 0.13	0.29; 0.05
PIIINP [ng/mL]	0.12; 0.34	0.35; 0.006	0.13; 0.37
OPN [ng/mL]	0.27; 0.02	0.37; 0.004	0.35; 0.005
TGF-β1 [pg/mL]	0.17; 0.17	−0.08; 0.51	0.05; 0.97
CTGF [ng/mL]	−0.09; 0.45	−0.04; 0.75	0.09; 0.53
MMP-2 [ng/mL]	0.27; 0.03	–	–
MMP-9 [pg/mL]	0.05; 0.68	–	–
TIMP-1 [pg/mL]	0.27; 0.03	–	–

r – Spearman rho correlation coefficient; PICP – carboxy-terminal propeptide of procollagen type I; PINP – amino-terminal propeptide of procollagen type I; PIIICP – carboxy-terminal propeptide of procollagen type III; PIIINP – amino-terminal propeptide of procollagen type III; OPN – osteopontin; TGF-β1 – transforming growth factor β1; CTGF – connective tissue growth factor; MMP-2 – matrix metalloproteinase-2; MMP-9 – matrix metalloproteinase-9; TIMP-1 – tissue inhibitor-1.

**Table 4.** Levels of galectin-3 at baseline and at 3- and 12-month follow-up in patients with new-onset and chronic DCM

Parameter	New-onset DCM (n = 35)	Chronic DCM (n = 35)	p-value
Baseline galectin-3 [ng/mL]	14.01 (11.17–17.8)	14.54 (11.04–16.39)	0.79
3-month galectin-3 [ng/mL]	12.42 (10.34–14.59)	12.66 (10.12–16.65)	0.53
12-month galectin-3 [ng/mL]	12.32 (10.22–15.26)	13.74 (10.43–16.59)	0.29

Data are presented as median and interquartile range; DCM – dilated cardiomyopathy.

**Table 5.** Levels of galectin-3 at baseline and at 3- and 12-month follow-up in DCM patients with and without ECM fibrosis

Parameter	Fibrosis negative DCM (n = 46)	Fibrosis positive (n = 24)	p-value
Baseline galectin-3 [ng/mL]	14.61 (11.46–17.47)	12.9 (10.33–16.18)	0.29
3-month galectin-3 [ng/mL]	12.58 (10.23–14.87)	12.18 (10.23–15.49)	0.94
12-month galectin-3 [ng/mL]	13.44 (10.28–16.17)	12.43 (10.89–14.38)	0.53

Data are presented as median and interquartile range.

had a tendency to increase between 3- and 12-months, at 12-months the levels still remained significantly lower in comparison to baseline values (14.61 ng/mL (11.46–17.47 ng/mL) compared to 13.44 ng/mL (10.28–16.17 ng/mL);  $p < 0.05$ ). The comparison of galectin-3 levels between 3- and 12-months showed similar results ( $p = 0.15$ ). Those patterns reveal that circulating galectin-3 decreases in DCM patients without fibrosis; in patients with fibrosis, galectin-3 levels are unchanged.

## Galectin-3 and CV outcomes in DCM

During the 12-month follow-up, CV death occurred in 6 (8.6%) patients and urgent HF hospitalization in 19 (27.1%) patients. Thus, the combined endpoint occurred in 25 patients. Cox proportional hazard analyses revealed that baseline galectin-3 was a predictor of CV death in unadjusted (hazard ratio (HR) = 1.204; 95% confidence interval (95% CI) = 1.024–1.415;  $p < 0.05$ ) and adjusted (HR = 1.246; 95% CI = 1.02–1.523;  $p < 0.05$ ) models. In addition, galectin-3

was also a significant predictor of the combined endpoint in unadjusted (HR = 1.105; 95% CI = 1.012–1.207;  $p < 0.05$ ) and adjusted (HR = 1.115; 95% CI = 1.009–1.231;  $p < 0.05$ ) models. The ROC analysis was conducted to identify the optimal galectin-3 level for the prediction of CV death and the combined endpoint. The optimal galectin-3 cut-off value for the prediction of CV death was 18.592 ng/mL, with a sensitivity of 66.7% and specificity of 84.4% (area under curve (AUC) = 0.74). Event rates were calculated using a Kaplan–Meier analysis according to the galectin-3 cut-off value determined with the ROC curve (Fig. 4). Patients with galectin-3  $\geq 18.592$  ng/mL had a significantly increased risk of CV death (HR = 5.053; 95% CI = 1.02–25.049;  $p < 0.05$ ) compared to those with galectin-3  $< 18.592$  ng/mL. The event curves are initially superimposed but begin to diverge after approx. 250 days. In terms of galectin-3 and the combined endpoint, the optimal cut-off value for galectin-3 was 14.54 ng/mL, with a sensitivity of 70.3% and specificity of 63% (AUC = 0.67). In addition, event rates were calculated according

to the galectin-3 cut-off of 14.54 ng/mL (Fig. 5). Patients with galectin-3  $\geq 14.54$  ng/mL had a significantly increased risk of the combined endpoint (HR = 2.569; 95% CI = 1.098–6.009;  $p < 0.05$ ) compare to those with galectin-3  $< 14.54$  ng/mL. The event curves diverge quickly at approx. 50 days and continue to diverge with time.

## Discussion

### Relationships between galectin-3 and ECM fibrosis and serum markers of fibrosis

Based on numerous in vitro experiments, galectin-3 has been established as an important pro-fibrotic protein.<sup>4,5,16</sup> However, the question remains whether serum galectin-3 level is associated with cardiac fibrosis and consequently can be used as a reliable biomarker. To date, investigators have reported contradictory findings. Following long-term LV assist device (LVAD) therapy, Lok et al. observed an increase in myocardial fibrosis that paralleled an increase in the concentration of galectin-3. Although the authors did not perform robust statistical calculations on the associations between fibrosis and galectin-3, these findings highlight their potential link.<sup>10</sup> In contrast, Besler et al. observed relatively strong correlations ( $r = 0.63$ ) between myocardial galectin-3 and biopsy-proven ECM fibrosis in inflammatory DCM. Importantly, the authors did not observe any relationship between serum galectin-3 and fibrosis.<sup>17</sup> Our study extends this negative finding on the lack of association between ECM fibrosis and serum galectin-3 to non-inflammatory DCM (as no acute or chronic myocardial inflammation was observed in myocardial samples).

The ECM fibrosis can also be assessed non-invasively by means of magnetic resonance imaging. Vergaro et al. observed that serum galectin-3 was an independent predictor of LV fibrosis as assessed by LGE in DCM patients.<sup>11</sup> In a similar fashion, Lepojärvi et al. found that patients with stable coronary artery disease who had the highest values of plasma galectin-3 had the lowest post-LGE T1 relaxation time, which was used as a non-invasive marker of cardiac fibrosis.<sup>18</sup> There are both advantages and disadvantages to determining fibrosis via biopsy or resonance. Nevertheless, our study clearly demonstrated the absence of a relationship between biopsy-proven fibrosis and circulating galectin-3.

Another approach used to estimate cardiac fibrosis is the measurement of circulating markers of fibrosis. However, this strategy should be interpreted with caution, as numerous studies have demonstrated the absence of associations between various markers of fibrosis and invasively determined fibrosis.<sup>15,19–21</sup> We report weak to moderate correlations between baseline galectin-3 and markers of collagen synthesis such as PIIICP, OPN, MMP-2, and TIMP-1. Uniquely, we investigated associations between

galectin-3 and markers of collagen synthesis, OPN, TGF, and CTGF at 3 and 12 months. In terms of markers of collagen synthesis, the observed pattern is not consistent as we observed changing correlations between markers of collagen type I and III synthesis. On the other hand, for fibrosis controlling factors, the pattern is clear, as galectin-3 repeatedly correlated with OPN.

### Kinetics of galectin-3 in dilated cardiomyopathy

Despite extensive exploration of galectin-3 in various cardiac conditions, the kinetics of galectin-3 have not been well-defined in patients with and without ECM fibrosis. As suggested in a recent American Heart Association (AHA) document, galectin-3 is a promising marker, but knowledge gaps – such as kinetic patterns – need to be addressed.<sup>22</sup>

Before any attempt is made to analyze the kinetics of any circulating marker, it is of paramount importance to understand its reference intervals, variability and biologic determinants. Krintus et al. have recently established galectin-3 reference intervals based on a study of 180 healthy individuals. They reported the lack of impact of known biological determinants, including age, on galectin-3 blood measurements.<sup>23</sup> In terms of HF and galectin-3, it was reported that galectin-3 short-term biologic variability, defined as 5 measurements within a three-week period, was 7.1%, and long-term (3 measurements within a three-month period) was 7.7%.<sup>24</sup> Similar observations were made by Meijers et al.; there were low levels of biologic variability of galectin-3 in HF, in comparison to much higher variability of NT-proBNP, over a six-week period.<sup>25</sup> In addition, we have previously reported the 12-month kinetics of serum markers of collagen synthesis, TGF and CTGF, which differ between patients with and without ECM fibrosis.<sup>26</sup> In the present study, the kinetics of galectin-3 were analyzed over a longer 3- and 12-month follow-up, and the observed values significantly exceeded previously reported biological variability. In terms of galectin-3 in patients with ECM fibrosis, repeated measurements are within expected biologic variability. We identified 2 distinct patterns of galectin-3 over a 12-month period. The 1<sup>st</sup> pattern was characterized by a gradual decrease and was observed in patients with a recent diagnosis of DCM and without ECM fibrosis. The 2<sup>nd</sup> pattern was characterized by stable measurements and was observed in patients with chronic DCM and with ECM fibrosis.

### Galectin-3 and outcomes in dilated cardiomyopathy

Despite numerous studies, the relationship between circulating galectin-3 and CV outcomes has not been definitively defined. Recently, professional cardiac societies

have suggested that galectin-3 is a potential new biomarker in the field of HF. However, the scientific evidence supporting the prognostic role of galectin-3 is weak and requires further evaluation. We identified studies with contradictory conclusions on the prognostic role of galectin-3 in HF.<sup>27–36</sup> Imran et al. have recently published a meta-analysis of 13 studies that evaluated the prognostic role of galectin-3 in the context of NT-proBNP and parameters of renal function.<sup>37</sup> The authors concluded that galectin-3 was independently associated with CV mortality, both in HF and in the general population. In our study, we do not attempt to conclusively define the relationship of galectin-3 role in HF given study limitations such as a small sample size and a short observational period. However, we studied a homogenous mid-sized DCM cohort and used well-defined, clinical endpoints of CV mortality and the combined endpoint of CV mortality and urgent HF hospitalization. We observed that galectin-3 was clearly associated with predefined endpoints. Based on ROC analyses, we were able to identify patients with a worse prognosis. Larger, well-designed studies are required to ultimately verify the prognostic role of galectin-3 before it can be included in diagnostic pathways.

## Limitations

We would like to acknowledge several limitations of the study. The proper assessment of fibrosis may be influenced by sampling error, patchy distribution of fibrosis, and harvesting from right ventricle (RV) compared to LV. The MMP-2, MMP-9 and TIMP-1 were measured only at baseline. Our study population of 70 patients may seem small, and the observation period of 1 year may be relatively short. On the other hand, all of our patients underwent biopsy, making the cohort at least a moderate-size population in the field of DCM and biopsy studies. During the 12-month follow-up, we observed the occurrence of the primary endpoint in 25 patients (6 deaths and 19 urgent hospital admissions), which constitutes a large number of events for analysis.

## Conclusions

Circulating galectin-3 is unrelated to invasively-determined ECM fibrosis in DCM. Serial measurements of galectin-3 correlated with markers of fibrosis, including markers of collagen synthesis and OPN. However, there were no correlations between galectin-3 and TGF or CTGF. Galectin-3 kinetics vary at 12 months in patients stratified according to disease duration and fibrosis status. Circulating galectin-3 was independently associated with CV outcomes in DCM.

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## References

- Weber KT. Cardiac interstitium in health and disease: The fibrillar collagen network. *J Am Coll Cardiol.* 1989;13(7):1637–1652. doi:10.1016/0735-1097(89)90360-4
- Japp AG, Gulati A, Cook SA, Cowie MR, Prasad SK. The diagnosis and evaluation of dilated cardiomyopathy. *J Am Coll Cardiol.* 2016;67(25):2996–3010. doi:10.1016/j.jacc.2016.03.590
- Gil CD, Cooper D, Rosignoli G, Perretti M, Oliani SM. Inflammation-induced modulation of cellular galectin-1 and -3 expression in a model of rat peritonitis. *Inflamm Res.* 2006;55(3):99–107.
- Sharma UC, Pokharel S, van Brakel TJ, et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation.* 2004;110(19):3121–3131. doi:10.1161/01.CIR.0000147181.65298.4D
- Calvier L, Martinez-Martinez E, Miana M, et al. The impact of galectin-3 inhibition on aldosterone-induced cardiac and renal injuries. *JACC Heart Fail.* 2015;3(1):59–67. doi:10.1016/j.jchf.2014.08.002
- Ho JE, Liu C, Lyass A, et al. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol.* 2012;60(14):1249–1256. doi:10.1016/j.jacc.2012.04.053
- De Boer RA, Voors AA, Muntendam P, Van Gilst WH, Van Veldhuisen DJ. Galectin-3: A novel mediator of heart failure development and progression. *Eur J Heart Fail.* 2009;11(9):811–817. doi:10.1093/eurjhf/hfp097
- Lok DJ, Van Der Meer P, de la Porte PW, et al. Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: Data from the DEAL-HF study. *Clin Res Cardiol.* 2010;99(5):323–328. doi:10.1007/s00392-010-0125-y
- López B, González A, Querejeta R, Zubillaga E, Larman M, Díez J. Galectin-3 and histological, molecular and biochemical aspects of myocardial fibrosis in heart failure of hypertensive origin. *Eur J Heart Fail.* 2015;17(4):385–392. doi:10.1002/ehf.246
- Lok SJ, Nous FM, van Kuik J, et al. Myocardial fibrosis and pro-fibrotic markers in end-stage heart failure patients during continuous-flow left ventricular assist device support. *Eur J Cardiothorac Surg.* 2015;48(3):407–415. doi:10.1093/ejcts/ezu539
- Vergaro G, Del Franco A, Giannoni A, et al. Galectin-3 and myocardial fibrosis in nonischemic dilated cardiomyopathy. *Int J Cardiol.* 2015;184:96–100. doi:10.1016/j.ijcard.2015.02.008
- Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: A position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J.* 2007;29(2):270–277. doi:10.1093/eurheartj/ehm342
- Cooper LT, Baughman KL, Feldman AM, et al; American Heart Association; American College of Cardiology; European Society of Cardiology; Heart Failure Society of America; Heart Failure Association of the European Society of Cardiology. The role of endomyocardial biopsy in the management of cardiovascular disease: A scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. *Circulation.* 2007;115(19):1914–1931. doi:10.1016/j.jacc.2007.09.008
- Rubiś P, Toton-Żurańska J, Wiśniowska-Śmiałek S, et al. Relations between circulating microRNAs (miR-21, miR-26, miR-29, miR-30 and miR-133a), extracellular matrix fibrosis and serum markers of fibrosis in dilated cardiomyopathy. *Int J Cardiol.* 2017;231:201–206. doi:10.1016/j.ijcard.2016.11.279



15. Rubiś P, Wiśniowska-Śmiałek S, Wypasek E, et al. Fibrosis of extracellular matrix is related to the duration of the disease but is unrelated to the dynamics of collagen metabolism in dilated cardiomyopathy. *Inflamm Res*. 2016;65(12):941–949. doi:10.1007/s00011-016-0977-3
16. Yu L, Ruifrok WP, Meissner M, et al. Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis. *Circ Heart Fail*. 2013;6(1):107–117. doi:10.1161/CIRCHEARTFAILURE.112.971168
17. Besler C, Lang D, Urban D, et al. Plasma and cardiac galectin-3 in patients with heart failure reflects both inflammation and fibrosis: Implications for its use as a biomarker. *Circ Heart Fail*. 2017;10(3):e003804. doi:10.1161/CIRCHEARTFAILURE.116.003804
18. Lepojärvi ES, Piira OP, Pääkkö E, et al. Serum PINP, PIIINP, galectin-3, and ST2 as surrogates of myocardial fibrosis and echocardiographic left ventricular diastolic filling properties. *Front Physiol*. 2015;6:200. doi:10.3389/fphys.2015.00200
19. Lopez B, Gonzalez A, Ravassa S, et al. Circulating biomarkers of myocardial fibrosis: The need for a reappraisal. *J Am Coll Cardiol*. 2015;65(22):2449–2456. doi:10.1016/j.jacc.2015.04.026
20. López-Andrés N, Rossignol P, Iraqi W, et al. Association of galectin-3 and fibrosis markers with long-term cardiovascular outcomes in patients with heart failure, left ventricular dysfunction, and dyssynchrony: Insights from the CARE-HF (Cardiac Resynchronization in Heart Failure) trial. *Eur J Heart Fail*. 2012;14(1):74–81. doi:10.1093/eurjhf/hfr151
21. Lin YH, Lin LY, Wu YW, et al. The relationship between serum galectin-3 and serum markers of cardiac extracellular matrix turnover in heart failure patients. *Clin Chim Acta*. 2009;409(1–2):96–99. doi:10.1016/j.cca.2009.09.001
22. Chow SL, Maisel AS, Anand I, et al; American Heart Association Clinical Pharmacology Committee of the Council on Clinical Cardiology; Council on Basic Cardiovascular Sciences; Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation; Council on Epidemiology and Prevention; Council on Functional Genomics and Translational Biology; and Council on Quality of Care and Outcomes Research. Role of biomarkers for the prevention, assessment, and management of heart failure: A scientific statement from the American Heart Association. *Circulation*. 2017;135(22):e1054–e1091. doi:10.1161/CIR.0000000000000490
23. Krintus M, Kozinski M, Fabiszak T, et al. Establishing reference intervals for galectin-3 concentrations in serum requires careful consideration of its biological determinants. *Clin Biochem*. 2017;50(10–11):599–604. doi:10.1016/j.clinbiochem.2017.03.015
24. Schindler EI, Szymanski JJ, Hock KG, Geltman EM, Scott MG. Short- and long-term biologic variability of galectin-3 and other cardiac biomarkers in patients with stable heart failure and healthy adults. *Clin Chem*. 2016;62(2):360–366. doi:10.1373/clinchem.2015.246553
25. Meijers WC, van der Velde AR, Muller Kobold AC, et al. Variability of biomarkers in patients with chronic heart failure and healthy controls. *Eur J Heart Fail*. 2017;19(3):357–365. doi:10.1002/ehfj.669
26. Rubiś P, Wiśniowska-Śmiałek S, Wypasek E, et al. 12-month patterns of serum markers of collagen synthesis, transforming growth factor and connective tissue growth factor are similar in new-onset and chronic dilated cardiomyopathy in patients both with and without cardiac fibrosis. *Cytokine*. 2017;96:217–227. doi:10.1016/j.cyt.2017.04.021
27. Wojciechowska C, Romuk E, Nowalany-Koziełska E, Jacheć W. Serum galectin-3 and ST2 as predictors of unfavorable outcome in stable dilated cardiomyopathy patients. *Hellenic J Cardiol*. 2017;58(5):350–359. doi:10.1016/j.hjc.2017.03.006
28. Hu DJ, Xu J, Du W, Zhang JX, Zhong M, Zhou YN. Cardiac magnetic resonance and galectin-3 level as predictors of prognostic outcomes for non-ischemic cardiomyopathy patients. *Int J Cardiovasc Imaging*. 2016;32(12):1725–1733. doi:10.1007/s10554-016-0958-1
29. Demissei BG, Cotter G, Prescott MF, et al. A multimarker multi-time point-based risk stratification strategy in acute heart failure: Results from the RELAX-AHF trial. *Eur J Heart Fail*. 2017;19(8):1001–1010. doi:10.1002/ehfj.749
30. Sygłłowicz G, Tomaniak M, Filipiak KJ, Kołowski Ł, Sitkiewicz D. Galectin-3 in patients with acute heart failure: Preliminary report on first Polish experience. *Adv Clin Exp Med*. 2016;25(4):617–623. doi:10.17219/acem/60527
31. Nymo SH, Aukrust P, Kjekshus J, et al; CORONA Study Group. Limited added value of circulating inflammatory biomarkers in chronic heart failure. *JACC Heart Fail*. 2017;5(4):256–264. doi:10.1016/j.jchf.2017.01.008
32. Wang CH, Cheng ML, Liu MH, Kuo LT, Shiao MS. Metabolic profile provides prognostic value better than galectin-3 in patients with heart failure. *J Cardiol*. 2017;70(1):92–98. doi:10.1016/j.jjcc.2016.10.005
33. Wang H, Chen Q, Li Y, Jing X, Liang T, Yang J. Prognostic value of galectin-3 on admission in Chinese patients with heart failure: A prospective observational study. *Acta Cardiol*. 2017;72(2):188–195. doi:10.1080/00015385.2017.1291187
34. McEvoy JW, Chen Y, Halushka MK, et al. Galectin-3 and risk of heart failure and death in Blacks and Whites. *J Am Heart Assoc*. 2016;5(5):e003079.
35. Sudharshan S, Novak E, Hock K, Scott MG, Geltman EM. Use of biomarkers to predict readmission for congestive heart failure. *Am J Cardiol*. 2017;119(3):445–451. doi:10.1016/j.amjcard.2016.10.022
36. Mueller T, Gegenhuber A, Leitner I, Poelz W, Haltmayer M, Dieplinger B. Diagnostic and prognostic accuracy of galectin-3 and soluble ST2 for acute heart failure. *Clin Chim Acta*. 2016;463:158–164. doi:10.1016/j.cca.2016.10.034
37. Imran TF, Shin HJ, Mathenge N, et al. Meta-analysis of the usefulness of plasma galectin-3 to predict the risk of mortality in patients with heart failure and in the general population. *Am J Cardiol*. 2017;119(1):57–64. doi:10.1016/j.amjcard.2016.09.019