IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Medical Sciences

2014; 11(11): 1098-1106. doi: 10.7150/ijms.8083

Research Paper

Comparison the Prognostic Value of Galectin-3 and Serum Markers of Cardiac Extracellular Matrix Turnover in Patients with Chronic Systolic Heart Failure

Yi-Yao Chang[1], Aaron Chen[2], Xue-Ming Wu[3], Tse-Pin Hsu[4], Li-Yu Daisy Liu[6], Yenh-Hsein Chen[4], Yen-Wen Wu[1,7], Hung-Ju Lin[4], Ron-Bin Hsu[5], Chi-Ming Lee[4], Shoei-Shen Wang[5], Men-Tzung Lo[8], Ming-Fong Chen[4], Yen-Hung Lin[4]✉

1. Cardiology Division of Cardiovascular Medical Center, Far Eastern Memorial Hospital, New Taipei City, Taiwan;
2. Woodhull Medical and Mental Hospital, Brooklyn, New York, USA;
3. Department of Internal Medicine, Taoyuan General Hospital, Taoyuan, Taiwan;
4. Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan;
5. Department of Surgery, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan;
6. Department of Agronomy, Biometry Division, National Taiwan University, Taipei, Taiwan;
7. National Yang-Ming University School of Medicine, Taipei, Taiwan;
8. Center for Dynamical Biomarkers and Translational Medicine, National Central University, Chungli, Taiwan.

✉ Corresponding author: Yen-Hung Lin, MD, PhD, Department of Internal Medicine, National Taiwan University Hospital, No.7, Chung-Shan S. Road, Taipei, 100, Taiwan (Tel: + 886-2-23123456 ext 62152/ FAX: + 886-2-23515811/ E-mail:austinr34@gmail.com).

Received: 2013.11.08; Accepted: 2014.07.31; Published: 2014.08.13

Abstract

Background: Galectin-3 (Gal-3) shows the ability of survival prediction in heart failure (HF) patients. However, Gal-3 is strongly associated with serum markers of cardiac extracellular matrix (ECM) turnover. The aim of this study is to compare the impact of Gal-3 and serum markers of cardiac ECM turnover on prognostic prediction of chronic systolic HF patients. **Methods**: Serum Gal-3, brain natriuretic peptide (BNP), extracellular matrix including type I and III aminoterminal propeptide of procollagen (PINP and PIIINP), matrix metalloproteinase-2, 9 (MMP-2, 9), and tissue inhibitor of metalloproteinase-1 (TIMP-1) were analyzed. Cox regression analysis was used for survival analysis.

Results: A total of 105 (81 male) patients were enrolled. During 980±346 days follow-up, 17 patients died and 36 episodes of HF admission happened. Mortality of these patients was significantly associated with the log PIIINP (β= 15.380; P=0.042), log TIMP-1(β= 44.530; P=0.003), log MMP-2 (β= 554.336; P<0.001), log BNP (β= 28.273; P=0.034). Log Gal-3 (β= 7.484; P=0.066) is borderline associated with mortality. Mortality or first HF admission of these patients was significantly associated with the log TIMP-1(β= 16.496; P=0.006), log MMP-2 (β= 221.864; P<0.001), log BNP (β= 5.999; P=0.034). Log Gal-3 (β= 4.486; P=0.095) only showed borderline significance. In several models adjusting clinical parameters, log MMP-2 was significantly associated with clinical outcome. In contrast, log Gal-3 was not.

Conclusion: The prognostic strength of MMP-2 to clinical outcome prediction in HF patients is stronger than Gal-3.

Key words: Gal-3, heart failure, MMP-2

Introduction

Heart failure (HF) is a disease causing high morbidity and mortality regardless of therapies [1]. Left ventricle (LV) remodeling plays a critical role in the progression of systolic HF. In previous studies, altered expressions of several serum markers of cardiac extracardiac matrix (ECM) turnover were recognized in patients with HF [2-4]. Furthermore, in some recent studies, the serum markers of cardiac ECM

turnover provide prognostic value and clinical implications in HF patients [2, 4-6]. Among these cardiac ECM markers, type III amioterminal propeptide of procollagen (PIIINP) is one of the most important ones. Serum PIIINP levels have been widely used to evaluate cardiac function [5], monitor exercise compacity and exercise tolerance [7], and predict prognosis in HF paitnets [5-7]. Matrix metalloproteinase-2 (MMP-2), which involves in the degradation of type IV collagen, is also a useful outcome predictor in patients with HF [4]. Besides, tissue inhibitor of metalloproteinase-1 (TIMP-1), which tightly regulates MMP activities, also has been shown to have prognostic implication in HF patients [8].

Inflammation pathway seems to play a significant role in development of HF [9-11]. Up to date, more and more evidence has linked macrophage activation and fibrosis to the pathogenesis of HF [10, 12], and galectin-3 (Gal-3) is one the most possible mediators [13]. Gal-3 is a member of the β-galactoside–binding animal lectin family, and its interaction with several ligands at the ECM, including laminin, synexin, integrins, and collagens, could modulate inflammation and immunity [14]. A previous study demonstrated that cardiac macrophages could produce Gal-3 after activation. Besides, the researchers also found the Gal-3– binding sites in cardiac fibroblasts and the ECM [15]. Moreover, intrapericardial infusion of recombinant Gal-3 in healthy rats led to LV systolic function impairement and increase of myocardial collagen content [15]. Among many mediators of interest, microarray studies have shown that Gal-3 is one of the most prominently mediators expressed in failing hearts [15].

Recently, several studies demonstrated the clinical prognostic value of Gal-3 in HF [16-23]. In our previous study in HF patients, we noticed significant relationships between Gal-3 and serum markers of cardiac ECM turnover including PIIINP, MMP-2 and TIMP-1 which also have significant clinical implication in HF outcome implication [24]. Therefore, a comparison among Gal-3 and serum markers of cardiac ECM turnover is important.

In this study, we tried to compare the impact of Gal-3 and serum markers of cardiac ECM turnover on prognostic prediction of chronic systolic HF patients. The primary goal is to predict all-cause mortality and the secondary goal is to predict all-cause mortality or time to first episode of HF hospitalization.

Methods

Patients

This is an extension study of our previous study in HF patients [24]. A total of 105 (81 males and 24 females) patients with chronic HF secondary to left ventricular systolic dysfunction (left ventricular ejection fraction ((LVEF)) ≤50% determined by echocardiography or Tc99m left ventriculography), who regularly visited the HF clinic in National Taiwan University Hospital, were enrolled in this study. Among then, 102 (97%) participated our previous study which was a cross section design to investigate the relation among Gal-3 and various fibrosis markers [24]. All patients received a full clinical history and examination performed by a cardiologist. Baseline demographic data, functional status, cardiovascular risk factors and medication were also recorded. The management of these heart failure patients was according to the guidelines of heart failure management [25]. Specialist nurse-led telephone visiting was conducted as our previous report [26]. The study was approved by the ethical committee of the National Taiwan University Hospital and all subjects gave informed consent in written form.

Laboratory analysis

Venous blood samples were collected after overnight fasting. After centrifugation, the serum was stored at −60 °C. Gal-3 was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Bender Medsystems, Vienna, Austria) on a Victor 2 plate reader (Perkin Elmer, Turku, Finland). The intra-assay variances of Gal-3 were 5.6%, and inter-assay were variances of Gal-3 was 8.6%. Brain natriuretic peptide (BNP) was measured by an ELISA kit (BNP-32, Phoenix pharmaceuticals, Belmont, USA). The intra-assay and inter-assay variation was <5% and <14%, respectively. Serum type I amioterminal propeptide of procollagen (PINP) was measured by a rapid equilibrium radioimmunoassay (RIA) kit (No. 67034, Orion Diagnostica, Espoo, Finland). The intra-assay and inter-assay variation s were both < 7%, and the detection limit was 2 μg/l. Serum PIIINP was determined by a coated-tube RIA method (No. 68570, Orion Diagnostica, Espoo, Finland). The intra- and inter-assay variations of serum PIIINP were both < 5%, and the detection limit was 0.3μg/l. TIMP-1 was measured by an ELISA kit (DTM100, R & D systems, Minneapolis, USA). The intra- and inter-assay variations of serum TIMP-1 were both < 5%, and the detection limit was 0.08 ng/ml. Serum MMP-2 was measured by an ELISA kit (DMP200, R & D systems, Minneapolis, USA). The intra- and inter-assay variations of this method were < 6% and <8%, respectively; the detection limit was 0.16 ng/ml [24].

Statistical analysis

Demographic data was presented as mean values ± standard deviations or as percentages. Data of

Gal-3, BNP, and serum cardiac ECM markers were presented as median and interquartile ranges due to non-normality which is tested by Kolmogorov-Smirnov test. These non-normal variables were log-transformed for further analysis. Pearson's correlation test was used to analyze the association between two variables.

Receiver operating characteristic (ROC) curves were performed and compared to estimate the prognostic capacity of Gal-3 and serum cardiac ECM markers. Furthermore, using the median value as cut-point, Kaplan-Meier survival curve with log-rank test was done to compare in patients with higher and lower levels of serum Gal-3 or cardiac ECM markers. Then, Cox regression analysis was used for survival analysis. A probability value of $p < 0.05$ was considered statistically significant and that of $0.05 < p < 0.1$ was considered as borderline significance. Statistical analyses were performed with SPSS for Windows, version 10.0 (SPSS Inc., Chicago, IL, USA).

Table 1. Clinical data of patients (n=105).

Patient characteristics	Data
Age (years)	62 ± 15
Male/Female	81/24
LVEF (%)	38 ± 11
NYHA Fc	2.1± 0.7
I/II/III/IV	18/59/28/0
Body weight (kg)	68 ± 16
Body height (cm)	165 ± 9
Body mass index (Kg/m²)	25± 4
Creatinine (mg/dL)	1.6 ± 1.3
Triglyceride (mg/dL)	156 ± 120
Cholesterol (mg/dL)	188 ± 46
HDL (mg/dL)	42± 9
LDL (mg/dL)	105 ± 40
WBC (/μl)	7.1 ± 2.4
Hemoglobin (g/dL)	13.5 ± 2.1
Etiology for heart failure	
Ischemic	46 (44)
Non-ischemic	59 (56)
Hypertension	49 (47)
Diabetes mellitus	29 (28)
Atrial fibrillation	31 (30)
Medication	
ACE-I	21 (20)
ARB	47 (45)
β-blocker	58 (55)
Loop diuretics	74 (70)
Digoxin	56 (53)
Spironolactone	33 (31)
BNP, pg/ml	2019 (1604-2541)
Gal-3, ng/ml	9.75 (7.08-12.69)
PINP, μg/L	33.8 (24.7-47.6)
PIIINP, μg/L	6.07 (4.74-7.19)
TIMP-1 ng/ml	135.8 (101.2-177.8)
MMP-2 ng/ml	256.5 (221.0-319.0)
MMP-9 ng/ml	48.0 (30.4-87.5)

Abbreviations: NYHA Fc= New York Heart Association functional classification; ACE-I= angiotensin converting enzyme inhibitor; ARB= angiotensin receptor blocker; BNP= brain natriuretic peptide; Gal-3= Galectin-3; PINP = type I amioterminal propeptide of procollagen; PIIINP = type III amioterminal propeptide of procollagen; TIMP = tissue inhibitor of metalloproteinase; MMP = matrix metalloproteinase.

Results

Baseline characteristics of HF patients

There were 105 (81 males and 24 females) patients participated in this study, and the mean age was 62 ± 15 years and LVEF was 38 ± 11%. Their mean NYHA was 2.1 and the patient numbers of NYHA I/II/III/IV were 18/59/28/0, respectively. Other clinical data, including Gal-3, serum cardiac ECM markers, and medication history were shown in Table 1. During 980±346 days follow-up, 17 patients died and 36 episodes of heart failure admission happened.

Correlations between Gal-3 and serum cardiac ECM markers

Log Gal-3 significantly correlated with log PIIINP (r=0.314, p=0.001), log TIMP-1 (r=0.286, p=0.003), log MMP2 (r=0.295, p=0.002), log BNP (0.234 p=0.016).

Prognostic value of Gal-3 and serum cardiac ECM markers for mortality

In ROC curve analysis for predicting mortality of patients (Figure 1), the area under the curve (AUC) of log MMP-2, log TIMP-1, log BNP, log PIIINP, log Gal-3, log PINP, and log MMP-9 were 0.786, 0.699, 0.636, 0.625, 0.607, 0.519, and 0.367 respectively.

Kaplan-Meier analysis of cumulative rates of survival was showed in Figure 2. The systolic HF patients presenting Gal-3 concentration lower than the median value, 9.75ng/ml, had non-significantly higher survival rate than those who had higher galectin-3 level (p=0.153). In contrast, patients presenting MMP-2 or TIMP-1 concentration lower than the median value had significantly higher survival rate than those who had higher MMP-2 or TIMP-1 level (p=0.001 for MMP-2 and p=0.028 for TIMP-1).

Mortality of these patients was significantly associated with the log PIIINP (β= 15.380; P=0.042), log TIMP-1(β= 44.530; P=0.003), log MMP-2 (β= 554.336; P<0.001), log BNP (β= 28.273; P=0.034), age (β= 1.075; P<0.001), and NYHA functional status (β= 6.301; P<0.001). Log Gal-3 (β= 7.484; P=0.066) and log MMP-9 (β= 0.212; P=0.073) only had borderline significance to the mortality (Table 2).

In the models adjusting clinical parameters (Table 3), log MMP-2 remained significance in five models. Log TIMP-1 remained significance in first three models, and became borderline significance in model 4 and 5. In contrast, log Gal-3 was not significantly associated with mortality in all five models. Log BNP was associated with mortality in model 1 and 5. Log MMP-9 was associated with mortality in model 5.

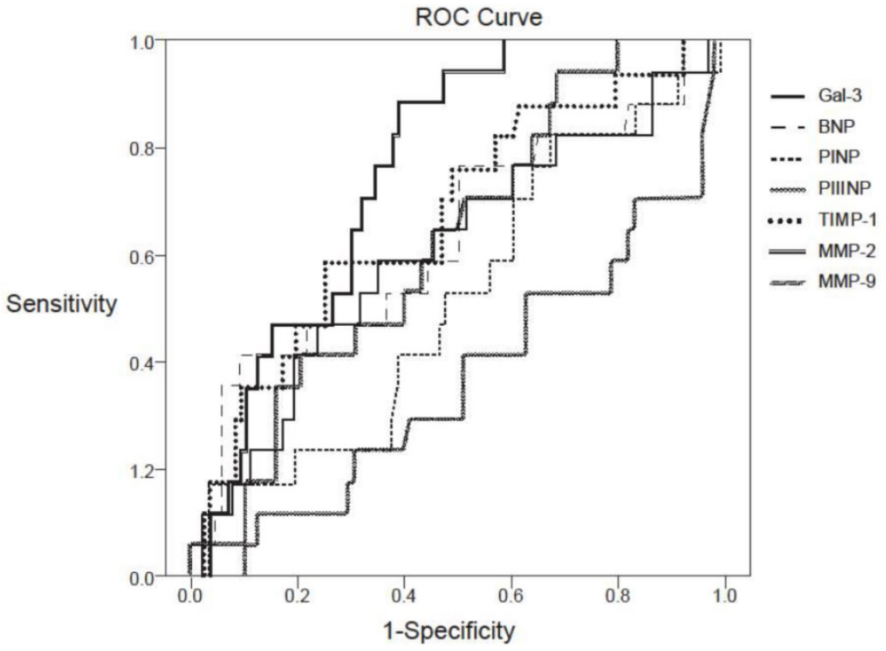

Figure 1. Receiver operating characteristic (ROC) curves for prediction mortality. The area under the curve (AUC) of log MMP-2, log TIMP-1, log BNP, log PIIINP, log Gal-3, log PINP, and log MMP-9 were 0.786, 0.699, 0.636, 0.625, 0.607, 0.519, and 0.367, respectively. Abbreviations: BNP= brain natriuretic peptide; Gal-3= Galectin-3; HF=heart failure; LVEF= left ventricular ejection fraction; MMP = matrix metalloproteinase; PINP = type I amioterminal propeptide of procollagen; PIIINP = type III amioterminal propeptide of procollagen; TIMP = tissue inhibitor of metalloproteinase.

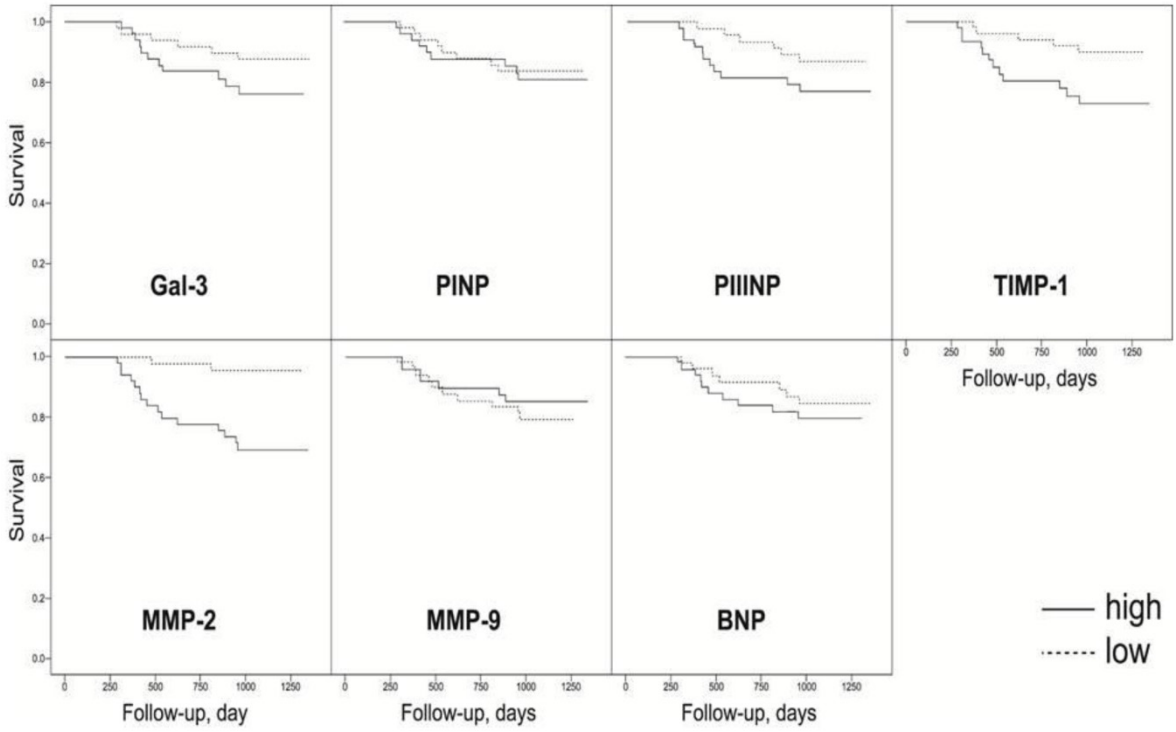

Figure 2. Kaplan-Meier analysis of cumulative rates of survival in HF patients with higher or lower levels of serum Gal-3 or cardiac ECM markers. The p value of Gal-3, PINP, PIIINP, TIMP-1, MMP-2, MMP-9, and BNP were 0.153, 0.708, 0.154, 0.028, 0.001, 0.501, and 0.483, respectively. Abbreviations: BNP= brain natriuretic peptide; ECM= Extracellular matrix; Gal-3= Galectin-3; HF=heart failure; LVEF= left ventricular ejection fraction; MMP = matrix metalloproteinase; PINP = type I amioterminal propeptide of procollagen; PIIINP = type III amioterminal propeptide of procollagen; TIMP = tissue inhibitor of metalloproteinase.

Table 2. Cox regression analysis for prediction of mortality using single variable.

	β (95% CI)	P value
Log Gal-3	7.484 (0.873, 64.156)	0.066
Log PINP	2.473 (0.216, 28.340)	0.467
Log PIIINP	15.380 (1.108, 213.582)	0.042
Log TIMP-1	44.530 (4.367, 454.056)	0.003
Log MMP-2	554.336 (16.800, 17637.141)	<0.001
Log MMP-9	0.212 (0.039, 1.159)	0.073
Log BNP	28.273 (1.282; 623.754)	0.034
Age	1.075 (1.034, 1.117)	<0.001
Sex, male	0.973 (0.317, 2.986)	0.973
Creatinine, mg/dl	1.173 (0.906, 1.520)	0.226
NYHA Fc	6.301 (2.483, 15.986)	<0.001
LVEF	0.996 (0.954, 1.039)	0.848
Usage of ACEI/ARB	1.110 (0.410, 3.002)	0.837
Usage ofβ-blocker	0.702 (0.271, 1.821)	0.467
Usage of spironolactone	0.825 (0.291, 2.344)	0.719
Usage of digoxin	0.980 (0.378, 2.541)	0.967
Ischemic origin of HF	1.459 (0.563, 3.783)	0.437
Hypertension	0.826 (0.315, 2.171)	0.699
Diabetes mellitus	2.381 (0.918, 6.172)	0.074

Abbreviations: ACE-I= angiotensin converting enzyme inhibitor; ARB= angiotensin receptor blocker; BNP= brain natriuretic peptide; Gal-3= Galectin-3; HF=heart failure; LVEF= left ventricular ejection fraction; MMP = matrix metalloproteinase; NYHA Fc= New York Heart Association functional classification; PINP = type I amioterminal propeptide of procollagen; PIIINP = type III amioterminal propeptide of procollagen; TIMP = tissue inhibitor of metalloproteinase.

Table 3. Cox regression analysis for prediction of mortality after adjusting clinical parameters.

		Log Galectin-3	Log PINP	Log PIIINP	Log TIMP-1	Log MMP-2	Log MMP-9	Log BNP
Model 1	β	5.587	4.892	14.141	96.433	1529.582	0.354	21.466
	p	0.138	0.225	0.059	0.002	0.002	0.196	0.028
Model 2	β	5.028	4.799	12.114	89.736	1367.862	0.380	18.599
	p	0.154	0.228	0.092	0.002	0.002	0.227	0.040
Model 3	β	5.048	4.790	11.154	109.041	1562.154	0.362	17.300
	p	0.152	0.223	0.102	0.002	0.002	0.205	0.053
Model 4	β	2.576	1.072	0.866	31.186	207.160	0.324	8.893
	p	0.381	0.959	0.939	0.052	0.027	0.169	0.102
Model 5	β	3.095	3.110	0.965	16.276	1320.064	0.138	35.427
	p	0.374	0.503	0.786	0.096	0.009	0.025	0.020

Model 1 adjusted by age.

Model 2 adjusted by age and sex.

Model 3 adjusted by age, sex, and LVEF.

Model 4 adjusted by age, sex, LVEF, and NYHA Fc.

Model 5 adjusted by age, sex, LVEF, creatinine, NYHA Fc, presence of hypertension, presence of diabetes mellitus, ischemic origin of HF, usage of ACEI or ARB, usage of β-blocker, usage of spironolactone, and usage of digoxin.

Abbreviations: ACE-I= angiotensin converting enzyme inhibitor; ARB= angiotensin receptor blocker; HF=heart failure; MMP = matrix metalloproteinase; NYHA Fc= New York Heart Association functional classification; PINP = type I amioterminal propeptide of procollagen; PIIINP = type III amioterminal propeptide of procollagen; TIMP = tissue inhibitor of metalloproteinase.

Prognostic value of Gal-3 and serum cardiac ECM markers for mortality or first HF admission

Kaplan-Meier analysis of cumulative rates of HF admission-free survival was shown in Figure 3. The systolic HF patients presenting Gal-3 concentration lower than the median value had non-significantly higher survival rate than those who had higher Gal-3 level (p=0.166). Patients presenting MMP-2 concentration lower than the median value had significantly higher survival rate than those who had higher MMP-2 level (p<0.001). Patients presenting TIMP-1 concentration lower than the median value had borderline significantly higher survival rate than those who had higher TIMP-1 level (p=0.085).

As shown in Table 4, mortality or first HF admission of these patients was significantly associated with the log TIMP-1(β= 16.496; P=0.006), log MMP-2 (β= 221.864; P<0.001), log BNP (β= 5.999; P=0.034), age (β= 1.063; P<0.001), and NYHA functional status (β= 4.370; P<0.001). Log Gal-3 (β= 4.486; P=0.095) and log MMP-9 (β= 0.313; P=0.079) only showed borderline significance.

In the models adjusting clinical parameters (Table 5), log MMP-2 remained significance in five models. Log TIMP-1 remained significance in first three models, and became no significance in model 4 and 5. In contrast, log Gal-3 was not significantly associated with mortality in all five models. Log MMP-9 was associated with mortality or first HF admission in model 5.

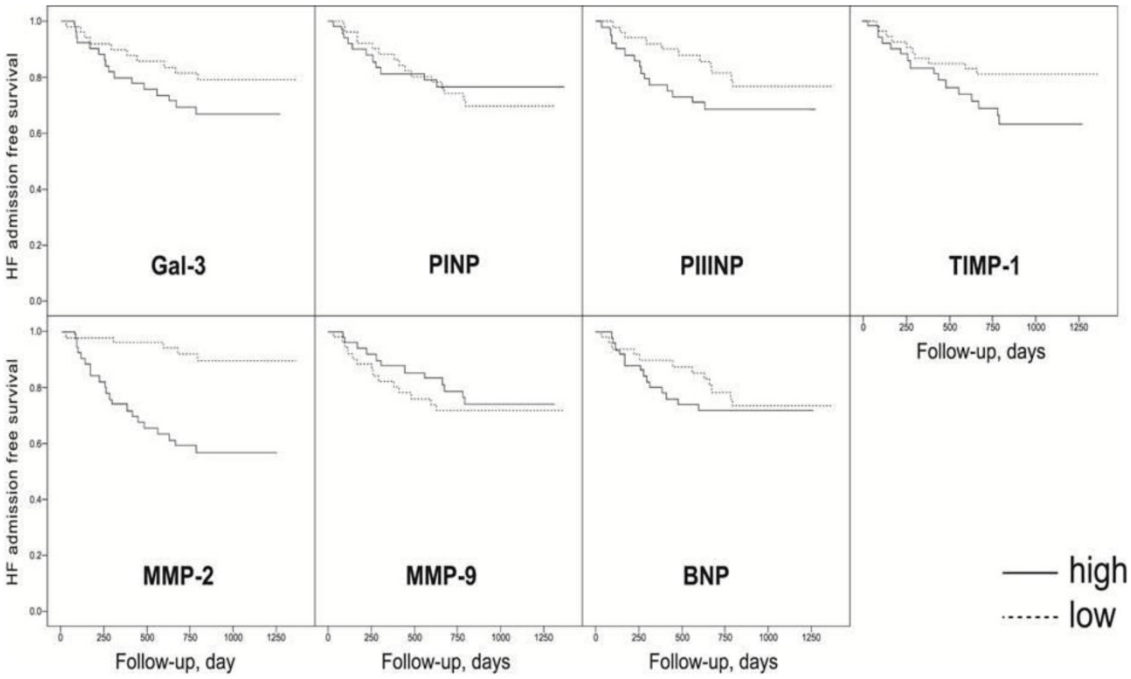

Figure 3. Kaplan-Meier analysis of cumulative rates of HF admission-free survival in HF patients with higher or lower levels of serum Gal-3 or cardiac ECM markers. The p value of Gal-3, PINP, PIIINP, TIMP-1, MMP-2, MMP-9, and BNP were 0.166, 0.624, 0.639, 0.085, <0.001, 0.624, and 0.684, respectively. Abbreviations: BNP= brain natriuretic peptide; ECM= Extracellular matrix; Gal-3= Galectin-3; HF=heart failure; MMP = matrix metalloproteinase; PINP = type I amioterminal propeptide of procollagen; PIIINP = type III amioterminal propeptide of procollagen; TIMP = tissue inhibitor of metalloproteinase.

Table 4. Cox regression analysis for prediction mortality or first HF admission using single variable.

	β (95% CI)	P value
Log Gal-3	4.486 (0.768; 26.191)	0.095
Log PINP	0.707 (0.093; 5.368)	0.737
Log PIIINP	8.278 (0.957; 71.603)	0.055
Log TIMP-1	16.496 (2.193; 124.066)	0.006
Log MMP-2	221.864 (13.477; 3652.544)	<0.001
Log MMP-9	0.313 (0.086; 1.143)	0.079
Log BNP	5.999 (0.460; 78.301)	0.034
Age	1.063 (1.031, 1.095)	<0.001
Sex, male	1.287 (0.485, 3.415)	0.612
Creatinine, mg/dl	1.171 (0.856, 1.603)	0.324
NYHA Fc	4.370 (2.189, 8.721)	<0.001
LVEF	1.010 (0.976, 1.045)	0.557
Usage of ACEI/ARB	0.751 (0.345, 1.626)	0.471
Usage of β-blocker	0.565 (0.259, 1.231)	0.151
Usage of spironolactone	0.577 (0.232, 1.436)	0.237
Usage of digoxin	0.775 (0.359, 1.677)	0.518
Ischemic origin of HF	1.902 (0.873, 4.142)	0.105
Hypertension	1.053 (0.487, 2.278)	0.895
Diabetes mellitus	2.099 (0.963, 4.572)	0.062

Abbreviations: ACE-I= angiotensin converting enzyme inhibitor; ARB= angiotensin receptor blocker; BNP= brain natriuretic peptide; Gal-3= Galectin-3; HF=heart failure; LVEF= left ventricular ejection fraction; MMP = matrix metalloproteinase; NYHA Fc= New York Heart Association functional classification; PINP = type I amioterminal propeptide of procollagen; PIIINP = type III amioterminal propeptide of procollagen; TIMP = tissue inhibitor of metalloproteinase.

Table 5. Cox regression analysis for prediction of mortality or first HF admission after adjusting clinical parameters.

		Log Galectin-3	Log PINP	Log PIIINP	Log TIMP-1	Log MMP-2	Log MMP-9	Log BNP
Model 1	β	3.639	1.132	5.465	14.906	198.288	0.392	7.082
	p	0.144	0.908	0.140	0.030	0.002	0.114	0.092
Model 2	β	3.348	1.091	3.651	13.081	182.747	0.414	5.692
	p	0.145	0.993	0.276	0.037	0.002	0.126	0.131
Model 3	β	3.444	1.081	3.875	12.749	179.315	0.419	6.385
	p	0.139	0.940	0.261	0.038	0.002	0.133	0.114
Model 4	β	2.279	0.485	0.708	2.711	48.807	0.389	4.333

	p	0.295	0.483	0.802	0.514	0.021	0.102	0.173
Model 5	β	4.455	0.359	0.331	1.729	101.504	0.210	5.606
	p	0.118	0.428	0.514	0.723	0.011	0.023	0.143

Model 1 adjusted by age

Model 2 adjusted by age and sex

Model 3 adjusted by age, sex, and LVEF

Model 4 adjusted by age, sex, LVEF, and NYHA Fc

Model 5 adjusted by age, sex, LVEF, creatinine, NYHA Fc, presence of hypertension, presence of diabetes mellitus, ischemic origin of HF, usage of ACEI or ARB, usage of β-blocker, usage of spironolactone, and usage of digoxin

Abbreviations: ACE-I= angiotensin converting enzyme inhibitor; ARB= angiotensin receptor blocker; HF=heart failure; MMP = matrix metalloproteinase; NYHA Fc= New York Heart Association functional classification; PINP = type I amioterminal propeptide of procollagen; PIIINP = type III amioterminal propeptide of procollagen; TIMP = tissue inhibitor of metalloproteinase.

Discussion

This study verified the values of these biomarkers in predicting clinical outcomes of HF. The results showed that the level of serum Gal-3 concentration had insignificant correlations to both overall and event-free survival, while serum MMP-2 showed better significance. This implicated that high level of serum Gal-3 could be a risk factor associating with outcomes of systolic HF patients and a likely clinically useful prognostic biomarker, but not as good as serum MMP-2. Furthermore, the predict ability of serum Gal-3 may be secondary to the predict ability of serum MMP-2 due to the high correlation between these factors.

Gal-3 has been thought as a potential biomarker for HF since Sharma et al observed that it is increased in decompensated heart failure in homozygous Ren-2 rats and published the first report in human subjects about increase of Gal-3 in the biopsies from patients with aortic stenosis with depressed ejection fraction [15, 27]. Van Kimmenade et al conducted the first clinical study and reported the value of Gal-3 in diagnosing acute HF and predicting short-term prognosis [21]. Moreover, analyzing the data from DEAL-HF study, Lok et al, further postulated that serum Gal-3 could be a novel prognostic marker in chronic HF patients [16].

Since then, more and more studies have investigated the correlations between Gal-3 and HF [17, 18, 28]. However, while many studies showed positive believes in Gal-3 as a novel biomarker, some have suggested modified opinions. For instance, defining HF with preserved ejection fraction, de Boer et al, concluded that Gal-3 could be an independent marker for outcome in HF but, in particularly, useful in HF patients with preserved LVEF [19]. In contrast, the predict ability was much lower in patients with HF with reduced LVEF. This result might explain the results of our study that Gal-3 had only borderline significance in predicting HF outcomes when we selected our patients with systolic HF with LVEF ≤ 50%.

Previous animal studies have demonstrated that increasing Gal-3 expressed by macrophages could activate fibroblasts via changes in expressions of cell cycle regulators, like cyclin D1, and induce fibrosis in different tissues, including lungs, kidneys and heart, which implies that Gal-3 might be a pro-fibrotic mediator in fibrosis process [16, 29, 30]. Since cardiac ECM markers, such as MMPs and TIMPs, regulate degrees of cardiac ECM turnover, the associations between Gal-3 and cardiac ECM markers would be significantly close due to their connections to fibrosis [31]. This explains the significant correlations between Gal-3 and cardiac ECM markers shown in our results. However, as a pro-fibrotic mediator, Gal-3 might have less association to the fibrosis, comparing with cardiac ECM markers, which directly regulate cardiac ECM integrity; therefore, prognostic strength of Gal-3 to the mortality might not be as strong as that of cardiac ECM markers.

MMP-2, MMP-9 and TIMP-1 have been demonstrated to contribute to ventricular remodeling and myocardial apoptosis in experimental pacing-induced HF model [32]. In clinical study, the circulating concentration and activity of MMP-2 and TIMP-1 also have been shown to be significantly correlated with HF development and have prognostic value in HF outcomes [3, 4, 8]. The results of our study further supported this observation. In addition, our study also showed that MMP-2 had higher significance than Gal-3 in predicting clinical outcome in systolic HF patients. However, in our study, MMP-2 had found to have significant correlations with clinical outcome in all models. In contrast, MMP-9 showed no significant relations with clinical outcome in unadjusted and four adjusted models (mole 1-4), which is similar to the results of Vorovich *et al* [33]. However, in model 5, MMP-9 was significantly correlated with clinical outcome after adjusting twelve clinical parameters. The prognostic value of MMP-9 for HF patients' needs further study.

PIIINP is another biomarker of interest in predicting HF outcomes. Several studies have suggested that PIIINP is an independent biomarker in predicting HF progression and associating with risks of worse outcomes [6, 34, 35]. From the CARE-HF trial, Natalia et al compared prognostic strength of Gal-3, PIIINP and MMP-1 [23]. They reported that increased Gal-3 and PIIINP in systolic HF patients were associated

with death or hospitalization, and MMP-1 ≤3ng/mL was associated with death or LVEF ≤35% at 18 months [23]. With less number of patients than CARE-HF trial, we observed Gal-3 and PIIINP only had borderline significant prognostic strengths to the HF outcomes, but MMP-2 and TIMP-1 were significantly better. Although our results support the observation that PIIINP or Gal-3 could be a prognostic biomarker of HF, it might also implicate that prognostic strength of MMP-2 is stronger than that of Gal-3 and PIIINP.

Gal-3 is an inflammatory mediator which induces and activates the progression of fibrosis. Gal-3 is also known to regulate many aspects of inflammatory cell behavior, and contributes to atherosclerotic plaque progression by enhancing monocyte chemoattraction through macrophage activation [36]. Gal-3 is also a marker of plague instability. In patients with coronary artery disease, unstable patients had a four-fold higher plasma Gal-3 levels in respect to the stable subjects regarding the left ventricular function [37]. In condition of HF, Gal-3 induces and activates the progression of fibrosis and play the role as a bridge connecting from inflammation to fibrosis. Therefore, plasma Gal-3 level may reflect the potential of further fibrosis process or cardiovascular event. In patients with acute coronary syndromes, higher concentration of Gal-3 is associated with risk of developing HF [18] and clinical events [38]. In Framingham Heart Study, higher concentration of Gal-3 is also associated with increased risk for incident HF and mortality in population without HF and very low prevalence of coronary artery disease [39]. In another study, Gal-3 predicts all-cause mortality in the general population [40].

In the relation among Gal-3 and fibrosis markers, Gal-3 is weakly but significantly correlated to type I collagen telopeptide (r = 0.27, P <0.0001), but not MMP-1 (r=-0.06, p=0.42), PIIINP (r=0.02, p-0.72), and PINP (r=0.11, p=0.12) in CARE-HF study [23]. In contrast, our finding is not like the data from CARE-HF. The association among Gal-3 and fibrosis markers is not fully studied in current stage. To our knowledge, only two studies reporting the relations (our previous study [24] and CARE-HF study). Several reasons may explain this situation. First, the racial difference may play a role. The Gal-3 levels are lower in Chinese than western countries in HF groups [23, 24]. In patients with acute coronary syndrome, another study done in Taiwan [38] also showed the same situation comparing with patients with acute coronary syndrome in TIMI-22 study [18]. Second, the different kits of testing biomarkers may cause difference. Third, difference of medication may also interfere the relation. The usage of angiotensin converting enzyme inhibitor/ angiotensin receptor blocker, beta-blocker and spironolactone is higher in CARE-HF than in the present study.

Several limitations were inherited in this study. This study has a relatively small patient number and is an extension study for our previous study. Small patient number may not enough to make the final conclusion and blunt the prognostic prediction ability of other serum markers such as BNP. Thus, a larger scale follow-up study is needed to validate the prognostic value of Gal-3 and other cardiac ECM markers. Next, there seems to have racial difference in Gal-3 levels [23, 24]. The low median level of Gal-3 could be an explanation of the moderate prognostic value of galectin-3 in this population. Therefore, a future study to verify the prognostic value of Gal-3 and other cardiac ECM markers should be performed in other races. Finally, a longer period of follow-up might be crucial to differentiate and ascertain prognostic value of each biomarker.

In conclusion, Gal-3 is significantly correlated with the serum markers of cardiac ECM turnover. The prognostic strength of MMP-2 to clinical outcome prediction in HF patients is stronger than Gal-3.

Acknowledgement

The authors would like to thank the staff of the Second Core Lab of Department of Medical Research and General Clinical Trial and Research in National Taiwan University Hospital for their great support. This study was supported by National Taiwan University Hospital (grants NTUH 100-M1708, NTUH 101-M1974, NTU 102-S2096), National Taiwan University (National Taiwan University Cutting-Edge Steering Research 10R71608-1), NTU-NTUH MediaTek Innovative Medical Electronics Research Center (PC851), Ministry of Science and Technology (NSC 102-2314-B-002-078-MY3, MOST 103-2220-E-002 -011), and Ministry of Science and Technology support for the Center for Dynamical Biomarkers and Translational Medicine, National Central University, Taiwan (NSC 101-2911-I-008-001, NSC 102-2911-I-008-001). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests

The authors have declared that no competing interest exists.

References

1 Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA *et al*. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur Heart J* 2008;29:2388-442.

2 Graham HK, Horn M and Trafford AW. Extracellular matrix profiles in the progression to heart failure. European Young Physiologists Symposium Keynote Lecture-Bratislava 2007. *Acta Physiol (Oxf)* 2008;194:3-21.

3 Yamazaki T, Lee JD, Shimizu H, Uzui H and Ueda T. Circulating matrix metalloproteinase-2 is elevated in patients with congestive heart failure. *Eur J Heart Fail* 2004;6:41-5.

4 George J, Patal S, Wexler D, Roth A, Sheps D and Keren G. Circulating matrix metalloproteinase-2 but not matrix metalloproteinase-3, matrix metalloproteinase-9, or tissue inhibitor of metalloproteinase-1 predicts outcome in patients with congestive heart failure. *Am Heart J* 2005;150:484-7.

5 Rossi A, Cicoira M, Golia G, Zanolla L, Franceschini L, Marino P *et al.* Amino-terminal propeptide of type III procollagen is associated with restrictive mitral filling pattern in patients with dilated cardiomyopathy: a possible link between diastolic dysfunction and prognosis. *Heart* 2004;90:650-4.

6 Cicoira M, Rossi A, Bonapace S, Zanolla L, Golia G, Franceschini L *et al.* Independent and additional prognostic value of aminoterminal propeptide of type III procollagen circulating levels in patients with chronic heart failure. *J Card Fail* 2004;10:403-11.

7 Radauceanu A, Ducki C, Virion JM, Rossignol P, Mallat Z, McMurray J *et al.* Extracellular matrix turnover and inflammatory markers independently predict functional status and outcome in chronic heart failure. *J Card Fail* 2008;14:467-74.

8 Frantz S, Stork S, Michels K, Eigenthaler M, Ertl G, Bauersachs J *et al.* Tissue inhibitor of metalloproteinases levels in patients with chronic heart failure: an independent predictor of mortality. *Eur J Heart Fail* 2008;10:388-95.

9 Reifenberg K, Lehr HA, Torzewski M, Steige G, Wiese E, Kupper I *et al.* Interferon-gamma induces chronic active myocarditis and cardiomyopathy in transgenic mice. *Am J Pathol* 2007;171:463-72.

10 Yndestad A, Ueland T, Oie E, Florholmen G, Halvorsen B, Attramadal H *et al.* Elevated levels of activin A in heart failure: potential role in myocardial remodeling. *Circulation* 2004;109:1379-85.

11 Bujak M and Frangogiannis NG. The role of IL-1 in the pathogenesis of heart disease. *Archivum immunologiae et therapiae experimentalis* 2009;57:165-76.

12 Weber KT, Gerling IC, Kiani MF, Guntaka RV, Sun Y, Ahokas RA *et al.* Aldosteronism in heart failure: a proinflammatory/fibrogenic cardiac phenotype. Search for biomarkers and potential drug targets. *Curr Drug Targets* 2003;4:505-16.

13 de Boer RA, Voors AA, Muntendam P, van Gilst WH and van Veldhuisen DJ. Galectin-3: a novel mediator of heart failure development and progression. *Eur J Heart Fail* 2009;11:811-7.

14 Ochieng J, Furtak V and Lukyanov P. Extracellular functions of galectin-3. *Glycoconj J* 2004;19:527-35.

15 Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B *et al.* Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation* 2004;110:3121-8.

16 Lok DJ, Van Der Meer P, de la Porte PW, Lipsic E, Van Wijngaarden J, Hillege HL *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;99:323-8.

17 McCullough PA, Olobatoke A and Vanhecke TE. Galectin-3: a novel blood test for the evaluation and management of patients with heart failure. *Rev Cardiovasc Med* 2011;12:200-10.

18 Grandin EW, Jarolim P, Murphy SA, Ritterova L, Cannon CP, Braunwald E *et al.* Galectin-3 and the development of heart failure after acute coronary syndrome: pilot experience from PROVE IT-TIMI 22. *Clin Chem* 2012;58:267-73.

19 de Boer RA, Lok DJ, Jaarsma T, van der Meer P, Voors AA, Hillege HL *et al.* Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. *Ann Med* 2011;43:60-8.

20 Tang WH, Shrestha K, Shao Z, Borowski AG, Troughton RW, Thomas JD *et al.* Usefulness of plasma galectin-3 levels in systolic heart failure to predict renal insufficiency and survival. *Am J Cardiol* 2011;108:385-90.

21 van Kimmenade RR, Januzzi JL, Jr., Ellinor PT, Sharma UC, Bakker JA, Low AF *et al.* Utility of amino-terminal pro-brain natriuretic peptide, galectin-3, and apelin for the evaluation of patients with acute heart failure. *J Am Coll Cardiol* 2006;48:1217-24.

22 Shah RV, Chen-Tournoux AA, Picard MH, van Kimmenade RR and Januzzi JL. Galectin-3, cardiac structure and function, and long-term mortality in patients with acutely decompensated heart failure. *Eur J Heart Fail* 2010;12:826-32.

23 Lopez-Andres N, Rossignol P, Iraqi W, Fay R, Nuee J, Ghio S *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;14:74-81.

24 Lin YH, Lin LY, Wu YW, Chien KL, Lee CM, Hsu RB *et al.* The relationship between serum galectin-3 and serum markers of cardiac extracellular matrix turnover in heart failure patients. *Clinica chimica acta; international journal of clinical chemistry* 2009;409:96-9.

25 Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG *et al.* ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. *Circulation* 2005;112:e154-235.

26 Ho YL, Hsu TP, Chen CP, Lee CY, Lin YH, Hsu RB *et al.* Improved cost-effectiveness for management of chronic heart failure by combined home-based intervention with clinical nursing specialists. *J Formos Med Assoc* 2007;106:313-9.

27 de Boer RA, Yu L and van Veldhuisen DJ. Galectin-3 in cardiac remodeling and heart failure. *Curr Heart Fail Rep* 2010;7:1-8.

28 Felker GM, Fiuzat M, Shaw LK, Clare R, Whellan DJ, Bettari L *et al.* Galectin-3 in Ambulatory Patients With Heart Failure: Results From the HF-ACTION Study. *Circ Heart Fail* 2012;5:72-8.

29 Kasper M and Hughes RC. Immunocytochemical evidence for a modulation of galectin 3 (Mac-2), a carbohydrate binding protein, in pulmonary fibrosis. *J Pathol* 1996;179:309-16.

30 Lin HM, Pestell RG, Raz A and Kim HR. Galectin-3 enhances cyclin D(1) promoter activity through SP1 and a cAMP-responsive element in human breast epithelial cells. *Oncogene* 2002;21:8001-10.

31 Li YY, McTiernan CF and Feldman AM. Interplay of matrix metalloproteinases, tissue inhibitors of metalloproteinases and their regulators in cardiac matrix remodeling. *Cardiovasc Res* 2000;46:214-24.

32 Song YH, Cai H, Gu N, Qian CF, Cao SP and Zhao ZM. Icariin attenuates cardiac remodelling through down-regulating myocardial apoptosis and matrix metalloproteinase activity in rats with congestive heart failure. *J Pharm Pharmacol* 2011;63:541-9.

33 Vorovich EE, Chuai S, Li M, Averna J, Marwin V, Wolfe D *et al.* Comparison of matrix metalloproteinase 9 and brain natriuretic peptide as clinical biomarkers in chronic heart failure. *Am Heart J* 2008;155:992-7.

34 Lin YH, Lin C, Lo MT, Lin HJ, Wu YW, Hsu RB *et al.* The relationship between aminoterminal propeptide of type III procollagen and heart rate variability parameters in heart failure patients: a potential serum marker to evaluate cardiac autonomic control and sudden cardiac death. *Clin Chem Lab Med* 2010;48:1821-7.

35 Zannad F, Alla F, Dousset B, Perez A and Pitt B. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evaluation study (RALES). Rales Investigators. *Circulation* 2000;102:2700-6.

36 Papaspyridonos M, McNeill E, de Bono JP, Smith A, Burnand KG, Channon KM *et al.* Galectin-3 is an amplifier of inflammation in atherosclerotic plaque progression through macrophage activation and monocyte chemoattraction. *Arteriosclerosis, thrombosis, and vascular biology* 2008;28:433-40.

37 Falcone C, Lucibello S, Mazzucchelli I, Bozzini S, D'Angelo A, Schirinzi S *et al.* Galectin-3 plasma levels and coronary artery disease: a new possible biomarker of acute coronary syndrome. *International journal of immunopathology and pharmacology* 2011;24:905-13.

38 Tsai TH, Sung PH, Chang LT, Sun CK, Yeh KH, Chung SY *et al.* Value and Level of Galectin-3 in Acute Myocardial Infarction Patients Undergoing Primary Percutaneous Coronary Intervention. *Journal of atherosclerosis and thrombosis* 2012;19(12):1073-82.

39 Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS *et al.* Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol* 2012;60:1249-56.

40 de Boer RA, van Veldhuisen DJ, Gansevoort RT, Muller Kobold AC, van Gilst WH, Hillege HL *et al.* The fibrosis marker galectin-3 and outcome in the general population. *Journal of internal medicine* 2012;272:55-64.