Chapter 6

Clinical Correlations of Plasma Galectin-3 Levels in a Well-defined Chronic Heart Failure Cohort

Lili Yu; Willem P.T. Ruifrok; Ysbrand T. Klip
Peter van der Meer MD, PhD; Wiek H. van Gilst
Rudolf A. de Boer

Manuscript

Abstract

Aims - Galectin-3 plays an important role in fibrogenesis. Furthermore, increased galectin-3 levels are associated with poor survival in patients with heart failure (HF). We examined the correlation of plasma galectin-3 levels with cardiopulmonary aerobic capacity and renal function in patients with chronic HF.

Methods and results - We measured plasma galectin-3 in 99 patients with stable chronic HF with New York Heart Association (NYHA) class II-IV. All patients had left ventricular ejection fraction (LVEF) ≤45% and an ability to undergo cardiopulmonary exercise testing. In the present HF cohort, plasma galectin-3 levels were divided in quartiles (quartile 1: <12.65 ng/mL; quartile 2: 12.65-14.34 ng/mL; quartile 3: 14.35-18.67 ng/mL; quartile 4: >18.67 ng/ml). High galectin-3 levels were associated with poor renal function (consisted of increased creatinin (p=0.026); increased urea (p=0.01); decreased eGFR (p=0.01), increased NT-proBNP (p=0.008), and decreased peak VO_2 (p=0.038). Linear regression analysis showed a correlation between the plasma galectin-3 levels and peak oxygen uptake (VO_2 max), p=0.016; and renal function (p=0.002). However, after adjustment for age and gender, the correlation between galectin-3 and VO_2 max and renal function was lost.

Conclusions - high plasma galectin-3 levels are associated with poor renal function and lower aerobic capacity in patients with chronic HF.

Key words

Chronic heart failure, Galectin-3, renal function, VO_2 max, cardiopulmonary aerobic capacity.

Introduction

Heart failure (HF) is a serious medical disease and an epidemiological problem. It is characterized by high morbidity and mortality [1-3]. The pathophysiologic mechanisms of HF appear to be the results of interaction between cardiac remodeling, neurohormonal peptides (e.g. N-terminal pro brain natriuretic peptide (NT-proBNP)), inflammation, and different biomarkers [4-6]. Accumulated experimental studies reported that macrophage-derived galectin-3 plays important regulatory roles in inflammation and fibrotic processes in the development cardiac remodeling and chronic HF [4-6]. Additionally, clinical evidence showed that plasma galectin-3 levels are increased in patients with acute and chronic HF [7-14]. The PRIDE study revealed that plasma galectin-3 was a superior predictor for 60-day mortality compared to NT-proBNP [11]. Subsequently, high plasma galectin-3 levels were associated with left ventricular filling and diastolic function [7]. Furthermore, in patients with chronic stable and acute decompensated HF, increased plasma galectin-3 levels were linked to renal dysfunction and lower peak oxygen uptake (VO_2 max) [8, 11, 15]. In the DEAL-HF study, plasma galectin-3 levels were increased in patients with higher NT-proBNP levels, which were in turn correlated with lower estimated glomerular filtration rate (eGFR) and lower VO_2 max [13]. Furthermore, higher levels of galectin-3 were found in patients with renal dysfunction as compared to patients with normal renal function. As a substantial part of chronic HF patients have decreased renal function, we examined the correlation of plasma galectin-3 levels on cardiopulmonary aerobic capacity and renal function in patients with chronic HF.

Methods

Patients and study design

The data described and used in this manuscript is derived from the BENEFICIAL study (Effects of alagebrium, an advanced glycation end product breaker, on exercise tolerance and cardiac function in patients with chronic heart failure, NTC00516646) [16-18]. The study design, baseline characteristics, inclusion and exclusion criteria have been published previously [16-18]. Data of all 99 patients who were recruited from the University Medical Center Groningen, the Netherlands and three other regional affiliated hospitals were analyzed in this sub study. Briefly, patients with New York Heart Association (NYHA) class II-IV had to have stable chronic HF for at least three months, and a documented left ventricular ejection fraction (LVEF) <45%. Main exclusion criteria were the inability of patients to undergo exercise testing, cardiac resynchronization therapy, pacemaker therapy, active and/or treated malignancies within 12 months prior to inclusion, and clinically significant renal dysfunction. The efficacy measurements included echocardiography and cardiopulmonary aerobic capacity testing. The BENEFICIAL study was approved by the Medical Ethical Committee of the University Medical Center Groningen and all subjects gave written informed consent.

Echocardiography

Two-dimensional echocardiography was performed by experienced cardiac technicians using a General Electric VIVID 7 system with a 2.5-3.5 MHz probe (Horton, Norway). Left ventricular dimensions were measured. Diastolic function was evaluated with peak early (E) and late (A) diastolic filling velocities, isovolumetric relaxation time (IVRT) and deceleration time (Dct) of the early peak filling. Early diastolic tissue velocity (E') was measured on the lateral and septal wall areas, using color-coded tissue Doppler imaging (CC-TDI). E/E' was calculated by dividing the peak early diastolic filling (E) by the average E'. Diastolic dysfunction was defined as an E/E' >10. Systolic dysfunction was determined by Simpson's LVEF and defined as a LVEF ≤45%. If Simpson's LVEF could not be determined, LVEF was estimated by eyeballing [16-18].

Cardiopulmonary aerobic capacity testing

Cardiopulmonary aerobic capacity testing was performed using a care fusion, Master screen CPX (Houten, The Netherlands) according to a modified Bruce protocol [19], which increases the workload more gradually than the Bruce protocol [20]. The first stage was performed at 1.7 mph and 0% grade, the second stage at 1.7 mph and 5% grade, and the third stage corresponds to the first stage of the Bruce protocol. A standard 12 lead electrocardiogram was recorded continuously during exercise testing. Blood pressure was registered on a regulatory basis using a manual cuff sphygmomanometer. Patients were encouraged to continue the exercise until their peak oxygen uptake (denoted as VO_2 max) was reached or when they became symptomatic, or discontinuation was indicated for safety reasons. Oxygen uptake, carbon dioxide production, and minute ventilation were measured using breath-by-breath gas analysis. Peak VO_2 was determined as an average value of the two highest VO_2 values at peak performance, data were expressed as mL/kg/min [16-18].

Biochemical measurements

Plasma Galectin-3 levels were determined by an enzyme-linked immunosorbent assay (ELISA) developed by BG Medicine (Galectin-3 assay^{TM}, BG Medicine, Inc., Waltham, USA). The assay quantitatively measures the concentration of human galectin-3 levels in EDTA plasma. This assay has a high sensitivity (lower limit of detection 1.13 ng/mL) and exhibits no cross-reactivity with collagens or other members of the galectin family. Calibration of the assay was performed according to the manufacturer's recommendation and values were normalized to a standard curve [9, 10]. NT-proBNP levels were measured by an immuno-electro-chemiluminesence method (Elecsys, Roche Diagnostics, Basel, Switzerland) [21]. The eGFR was estimated using the simplified modification of Diet in Renal Disease (sMDRD) formula [22].

Statistical analysis

Data are expressed as mean ± standard deviation (SD) when normally distributed. Data are expressed as medians with lower and upper quartiles when non-normally distributed.

Categorial variables are expressed as frequencies and percentages. Baseline characteristics were divided into quartiles of plasma galectin-3 levels. Differences between groups were compared using the 1-way analysis of variance test, Kruskal-Wallis test or Chi-square test where appropriate. For further analyses, logarithmic transformation was performed to achieve a normal distribution for skewed variables. The Pearson correlation coefficient was employed to test correlations between galectin-3 and other variables.

All tests were two-sided and a p-value <0.05 was considered statistically significant. All statistical analyses were performed using STATA version 11.0 (StataCorp LP) and SPSS version 18.0 (SPSS Inc).

Results

Patient characteristics

Baseline characteristics of all patients, according to quartiles of plasma galectin-3, are described in table 1. Overall, mean age of the study population was 61±11 years, and 80% were males. Around 40% patients had NYHA-class III and IV. Mean LVEF was 32±9%. Mean eGFR was 80±21 mL/min/1.73m^2, mean NT-proBNP value was 388 (154-823) ng/L, mean VO_2 max was 21.7±6.1 mL/kg/min. All patients were on standard medication for HF, including an ACE inhibitor (ACEi) or angiotensin II receptor blocker (ARB), beta-blocker (BB), and diuretics.

Galectin-3 and parameters of disease severity and renal function

Plasma galectin-3 levels displayed a moderately significant correlation with levels of NT-proBNP (R=0.26; p=0.009; figure 1A), but not with NYHA-class (R=0.13; p=0.230). Furthermore, galectin-3 levels also showed a correlation with creatinin levels (R=0.24; p=0.014) and with levels of plasma urea (R=0.33; p=0.001). In addition, linear regression analysis showed a significant association between plasma galectin-3 levels and eGFR (R=-0.30; p=0.002; figure 1B). When adjusted for age and gender plasma galectin-3 levels are not correlated with eGFR, suggesting that some of the prognostic power of galectin-3 may be associated with age and gender.

Correlation between plasma galectin-3 levels and exercise capacity

At baseline, VO_2 max correlated with increasing galectin-3 levels (R=-0.24; p=0.016; figure 1C). In contrast, the level of plasma galectin-3 is not associated with the resting oxygen uptake (R=-0.11; p=0.285). Furthermore, linear regression analysis showed a correlation between the plasma galectin-3 levels and the VO_2 max (p=0.016). However, when corrected for age and gender, plasma galectin-3 is no longer correlated with VO_2 max (figure 1).

Correlation between plasma galectin-3 and echocardiographic parameters

Table 1. Baseline parameters according to the plasma galectin-3 levels

		Quartiles of galectin-3				
Variables	All patients (n=99)	Quartile 1 (<12.65 ng/mL)	Quartile 2 (12.65-14.34 ng/mL)	Quartile 3 (14.35-18.67 ng/mL)	Quartile 4 (>18.67 ng/mL)	p-value for trend
Age, years	61 ± 11	59 ± 11	58 ± 9	63 ± 12	61 ± 11	0.085
Sex, male, %	80	92	84	80	64	0.100
Ischemic cause of HF, %	70	75	72	52	80	0.148
BMI, kg/m^2	28±4	28±3	28±4	28±5	27±4	0.905
NYHA class, %, II/III/IV	64/33/3	71/29/0	58/40/2	72/28/0	56/36/8	0.512
SBP, mmHg	115±15	113±12	115±14	116±17	115±17	0.944
DBP, mmHg	72±9	73±9	71±8	75±11	70±9	0.231
Heart rate, bpm	69±14	71±17	64±14	72±11	71±12	0.101
Co-morbidities (%)						
Hypertension	31	33	24	32	36	0.819
Hypercholesterolemia	64	83	68	56	48	0.048
Diabetes	16	21	8	16	20	0.596
Laboratory						
Hb, mmol/L	8.9±0.8	9.0±0.6	9.2±0.7	8.8±0.9	8.8±0.8	0.328
Sodium, mmol/L	140±2	140±2	140±2	140±2	141±2	0.766
Potassium, mmol/L	4.2±0.3	4.3±0.3	4.2±0.3	4.2±0.4	4.1±0.4	0.562
Total cholesterol, mmol/L	4.4±1.2	4.2±0.9	4.5±0.9	4.6±1.3	4.3±1.5	0.762
Ferritin, µg/L	128 (71-206)	117 (59-296)	173 (121-225)	126 (86-181)	111 (49-150)	0.053
TSAT, %	31 (25-39)	32 (23-40)	32 (27-39)	35 (28-41)	27 (19-36)	0.278
sTfR, mg/L	1.21 (1.02-1.49)	1.14(1.03-0.48)	1.21 (0.97-1.33)	1.22 (0.99-1.55)	1.25 (1.11-1.62)	0.672
Creat, µmol/L	93±25	90±20	83±18	93±28	104±26	0.026
Urea, mmol/L	7.6±2.9	7.1±2.8	6.5±1.7	7.6±2.8	9.2±3.4	0.010
eGFR, ml/min/1.73m^2	80±21	84±13	87±21	80±22	69±21	0.010
NT-proBNP, ng/L	388(154-823)	312(187-655)	199(94-380)	463(154-1060)	499(307-1706)	0.008
hs-CRP, mg/L	1.6 (0.8-3.7)	1.4 (0.9-2.6)	1.3 (0.8-3.7)	2.1 (0.8-4.5)	2.1 (0.9-3.7)	0.589
Treatment (%)						
ACEi	79	71	80	80	84	0.715

ARB	17	29	12	20	8	0.211
BB	93	100	88	92	92	0.421
Diuretics	60	46	52	64	76	0.140
Exercise test						
Resting VO2, ml/min	357±81	370±86	346±71	377±93	337±69	0.251
VO2max, ml/kg/min	21.7±6.1	23.6±5.8	23±7.2	21.3±4.7	19.2±6.0	0.038
RQ	1.06±0.09	1.10±0.09	1.07±0.09	1.05±0.09	1.01±0.06	0.029
Echo parameters						
LVEDD, mm	59(54-62)	60(55-62)	58(48-62)	59(57-62)	57(53-63)	0.665
LVESD, mm	47(41-53)	59(42-53)	46(41-52)	47(43-55)	44(39-54)	0.776
E/A ratio	0.89 (0.71-1.20)	0.84(0.70-1.30)	0.93 (0.68-1.29)	0.88 (0.65-1.17)	0.90 (0.73-1.03)	0.872
E/E' ratio	12.6 (10-18.3)	11.7 (9.5-15.8)	12.8 (9.5-17.6)	12.7 (10.3-18.2)	14.6 (11-23.9)	0.351
IVRT, ms	99 (83-116)	94 (89-118)	105 (89-126)	96 (83-107)	95 (83-107)	0.332
Dct, ms	209 (172-245)	219 (166-260)	195 (169-233)	190 (161-245)	210 (191-245)	0.662
TAPSE	19 (17-23)	20 (18-22)	19 (18-23)	19 (15-25)	20 (17-23)	0.804
Simpson LVEF, %	32±9	34±9	34±8	32±6	29±11	0.373
Eyeballing LVEF, %	32±10	33±9	34±9	30±9	31±12	0.467

HF: heart failure; BMI: body mass index; NYHA: New York Heart Association; SBP: systolic blood pressure; DBP: diastolic blood pressure; bpm: beats per minute; Hb: hemoglobin; TSAT: transferrin saturation; sTfR: serum transferrin receptor; creat: creatinin; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro brain natriuretic peptide; hs-CRP: high-sensitive C-reactive protein; ACEi: ACE-inhibitor; ARB: angiotenis II receptor blocker; BB: beta-blocker**;** RQ: respiration quotient**;** LVEDD: left ventricular end diastolic diameter; LVESD: left ventricular end systolic diameter; IVRT: isovolumetric relaxation time; Dct: deceleration time; LVEF: left ventricular ejection fraction; E': early diastolic tissue velocity; E; peak early filling velocity; A: late diastolic filling velocity.

We analyzed the relation between plasma galectin-3 and different echocardiographic parameters. The interactions are described in table 1. The results show that increased plasma galectin-3 levels are not associated with parameters of diastolic function (E/A, p=0.872; E/E', p=0.351; IVRT, p=0.332; Dct, p=0.662) or systolic function (Simpson LVEF%, p=0.373) (table 1). Linear regression analysis showed that there is no correlation between plasma galectin-3 and LVEF (R=-0.21; p=0.105; figure 1D).

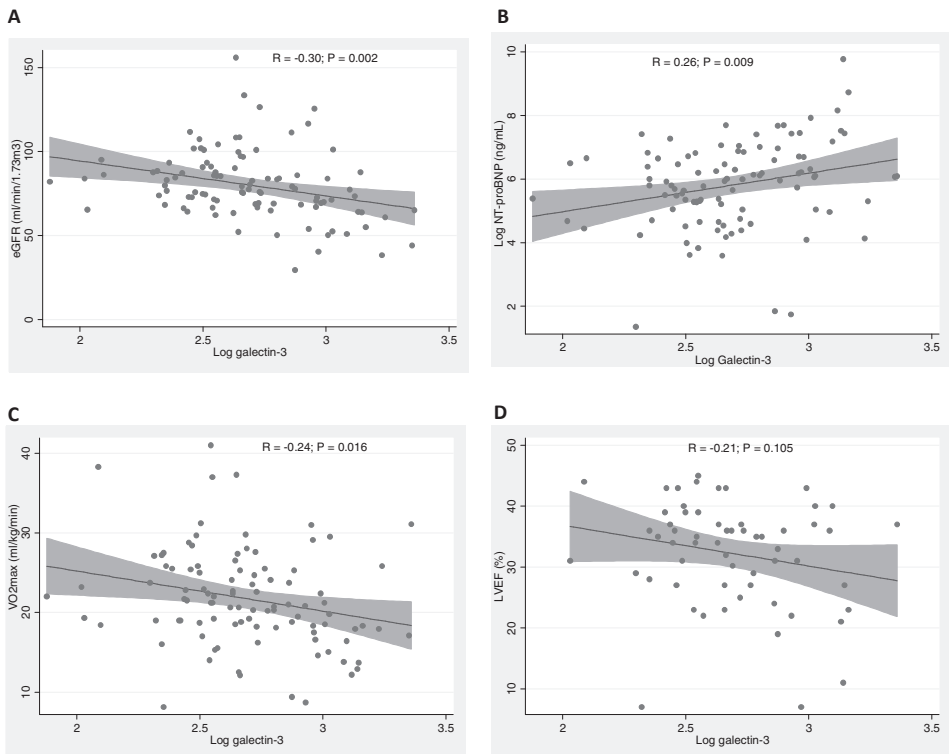

Figure 1 Univariate relation (and 95% confidence intervals) between log galectin-3 in HF patients and renal function (A), NT-proBNP (B), VO_2 max (C) and LVEF (D)

Discussion

The present data shows that if galectin-3 levels in patients with chronic HF are high at baseline, this is strongly associated with increased creatinin, increased urea and decreased eGFR. Furthermore, high galectin-3 levels at baseline are associated with increased levels of NT-proBNP. However, when corrected for age and gender, plasma galectin-3 shows no significant relation with eGFR.

We observed a strongly correlation between plasma galectin-3 and VO_2 max, however, this correlation is abrogated after correction for age and gender. Finally, no correlation was found between increased levels of galectin-3 and echocardiographic measurements of cardiac function in chronic HF.

Galectin-3 was discovered around ten years ago. It is widely distributed throughout the entire body, including heart, lung, liver and kidney [23]. The role of galectin-3 in fibrosis and inflammation has been elucidated in recent years. The first experimental evidence showing involvement of galectin-3 in chronic HF stems from a landmark study of Sharma and colleagues [4]. They demonstrated that galectin-3 could be used as a new target for intervention in chronic HF. Since then, clinical trials have consistently shown potential clinical usefulness of galectin-3 as a prognostic biomarker for chronic HF. Herein, van Kimmenade et al. were the first to evaluate the prognostic and predictive value of galectin-3 as a biomarker in acute and chronic HF [11].

The PRIDE study revealed that plasma galectin-3 was a superior predictor when compared to NT-proBNP. High plasma galectin-3 levels are associated with left ventricular filling and decreased diastolic function [7]. In contrast, in our study we found no relation between plasma galectin-3 levels and echocardiographic parameters for diastolic and systolic function in this cohort of patients with chronic HF. We argue that the discrepancy between previous reports and our study is due to fact that the patient's population in the PRIDE study is different from our present study (acutely decompensated HF in PRIDE vs. well defined stable chronic HF in the present study).

In the DEAL-HF study, plasma galectin-3 levels were increased in patients with higher NT-proBNP levels. Furthermore high galectin-3 levels were associated with decreased eGFR. Additionally, in the PRIDE and the PREVEND studies, our group showed a correlation between increased plasma galectin-3 levels and cardiovascular risk factors and renal dysfunction. Notably, growing evidence shows that renal dysfunction is frequently observed in cardiovascular disease [25, 26], being one of the most powerful predictors in chronic HF prognosis and plays an important role in the pathophysiologic process of HF [27]. Taken together, evidence is accumulating that increased plasma galectin-3 levels are associated with cardiovascular disease and renal dysfunction. We confirm these observations in our present study.

Interestingly, in a recently published paper by Gopal et al. plasma galectin-3 is inversely related to renal function in patients with and without clinical HF [28]. They showed that galectin-3 correlated strongly with eGFR, both in patients with HF and in patients without HF, and this relationship was unaffected by the presence or absence of clinical HF. They concluded that concentrations of plasma galectin-3 do not seem to depend on the level of compensation or type of HF. Furthermore, the relationship between galectin-3 and renal function seems to be affected little or not at all by the presence or absence of clinical HF.

Cardiopulmonary aerobic capacity testing is one of the diagnostic tools for chronic HF. Decreased peak exercise capacity is associated with poor prognostic and decreased patient

survival. In the HF-ACTION study, plasma galectin-3 levels were measured in 895 subjects with chronic HF from a randomized controlled trial of exercise training in patients with chronic HF (NYHA class II, III or IV). Galectin-3 was associated with increased NYHA-class, and lower VO_2 max [13]. The present data confirms that increased plasma galectin-3 levels are associated with decreased VO_2 max.

Some limitations apply to this study. This is a sub study of the BENEFICIAL study and the BENEFICIAL study was not powered for the current analyses. After correction for age and gender, plasma galectin-3 shows no significant relation with eGFR, VO_2 max and echocardiographic measurements of cardiac function, where previous studies do. This is probably due to the low number of subjects participating in this study.

As a multifunctional biomarker, galectin-3 promotes macrophage migration, myofibroblast activation and collagen synthesis, all involved in organ fibrogenesis process. The relationship of galectin-3 with other cardiovascular markers (e.g.: LVEF, NT-proBNP), renal function and VO_2 max suggests a role of galectin-3 in integrating these mechanisms in the progression of HF. However, still much is unknown about the role of galectin-3 in cardiovascular disease. Well-designed studies are needed to further elucidate its role in chronic HF.

Conclusions

This study demonstrates that high plasma galectin-3 levels are correlated with poor renal function and lower aerobic capacity in patients with chronic HF. No significant relation was observed between plasma galectin-3 levels and echocardiographic parameters for chronic HF. Although much about the details of galectin-3 in HF remains vague, continued efforts at increasing precision may uncover a new chapter in our understanding of HF pathophysiology.

References

1. McMurray JJ, Teerlink JR, Cotter G, Bourge RC, Cleland JG, Jondeau G, Krum H, Metra M, O'Connor CM, Parker JD, Torre-Amione G, van Veldhuisen DJ, Lewsey J, Frey A, Rainisio M, Kobrin I, VERITAS Investigators (2007) Effects of tezosentan on symptoms and clinical outcomes in patients with acute heart failure: the VERITAS randomized controlled trials. JAMA 298:2009-2019

2. Jaarsma T, van der Wal MH, Lesman-Leegte I, Luttik ML, Hogenhuis J, Veeger NJ, Sanderman R, Hoes AW, van Gilst WH, Lok DJ, Dunselman PH, Tijssen JG, Hillege HL, van Veldhuisen DJ, Coordinating Study Evaluating Outcomes of Advising and Counseling in Heart Failure (COACH) Investigators (2008) Effect of moderate or intensive disease management program on outcome in patients with heart failure: Coordinating Study Evaluating Outcomes of Advising and Counseling in Heart Failure (COACH). Arch Intern Med 168:316-324

3. Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA, Stromberg A, van Veldhuisen DJ, Atar D, Hoes AW, Keren A, Mebazaa A, Nieminen M, Priori SG, Swedberg K, ESC Committee for Practice Guidelines (CPG) (2008) 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 J Heart Fail 10:933-989

4. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B, Andre S, Crijns HJ, Gabius HJ, Maessen J, Pinto YM (2004) Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. Circulation 110:3121-3128

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

6. de Boer RA, Voors AA, Muntendam P, van Gilst WH, van Veldhuisen DJ (2009) Galectin-3: a novel mediator of heart failure development and progression. Eur J Heart Fail 11:811-817

7. Shah RV, Chen-Tournoux AA, Picard MH, van Kimmenade RR, Januzzi JL (2010) Galectin-3, cardiac structure and function, and long-term mortality in patients with acutely decompensated heart failure. Eur J Heart Fail 12:826-832

8. Lok DJ, Van Der Meer P, de la Porte PW, Lipsic E, Van Wijngaarden J, Hillege HL, van Veldhuisen DJ (2010) 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 99:323-328

9. de Boer RA, Lok DJ, Jaarsma T, van der Meer P, Voors AA, Hillege HL, van Veldhuisen DJ (2011) Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. Ann Med 43:60-68

10. de Boer RA, van Veldhuisen DJ, Gansevoort RT, Muller Kobold AC, van Gilst WH, Hillege HL, Bakker SJ, van der Harst P (2011) The fibrosis marker galectin-3 and outcome in the general population. J Intern Med

11. van Kimmenade RR, Januzzi JL,Jr, Ellinor PT, Sharma UC, Bakker JA, Low AF, Martinez A, Crijns HJ, MacRae CA, Menheere PP, Pinto YM (2006) 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 48:1217-1224

12. Christenson RH, Duh SH, Wu AH, Smith A, Abel G, deFilippi CR, Wang S, Adourian A, Adiletto C, Gardiner P (2010) Multi-center determination of galectin-3 assay performance characteristics: Anatomy of a novel assay for use in heart failure. Clin Biochem 43:683-690

13. Felker GM, Fiuzat M, Shaw LK, Clare R, Whellan DJ, Bettari L, Shirolkar SC, Donahue M, Kitzman DW, Zannad F, Pina IL, O'Connor CM (2012) Galectin-3 in ambulatory patients with heart failure: results from the HF-ACTION study. Circ Heart Fail 5:72-78

14. Grandin EW, Jarolim P, Murphy SA, Ritterova L, Cannon CP, Braunwald E, Morrow DA (2012) Galectin-3 and the development of heart failure after acute coronary syndrome: pilot experience from PROVE IT-TIMI 22. Clin Chem 58:267-273

15. Lainscak M, Coletta AP, Sherwi N, Cleland JG (2010) Clinical trials update from the Heart Failure Society of America Meeting 2009: FAST, IMPROVE-HF, COACH galectin-3 substudy, HF-ACTION nuclear substudy, DAD-HF, and MARVEL-1. Eur J Heart Fail 12:193-196

16. Hartog JW, Willemsen S, van Veldhuisen DJ, Posma JL, van Wijk LM, Hummel YM, Hillege HL, Voors AA, BENEFICIAL investigators (2011) Effects of alagebrium, an advanced glycation endproduct breaker, on exercise tolerance and cardiac function in patients with chronic heart failure. Eur J Heart Fail 13:899-908

17. Willemsen S, Hartog JW, Hummel YM, Posma JL, van Wijk LM, van Veldhuisen DJ, Voors AA (2010) Effects of alagebrium, an advanced glycation end-product breaker, in patients with chronic heart failure: study design and baseline characteristics of the BENEFICIAL trial. Eur J Heart Fail 12:294-300

18. Willemsen S, Hartog JW, Hummel YM, van Ruijven MH, van der Horst IC, van Veldhuisen DJ, Voors AA (2011) Tissue advanced glycation end products are associated with diastolic function and aerobic exercise capacity in diabetic heart failure patients. Eur J Heart Fail 13:76-82

19. Sheffield LT, Roitman D (1976) Stress testing methodology. Prog Cardiovasc Dis 19:33-49

20. van den Broek SA, van Veldhuisen DJ, de Graeff PA, Landsman ML, Hillege H, Lie KI (1992) Comparison between New York Heart Association classification and peak oxygen consumption in the assessment of functional status and prognosis in patients with mild to moderate chronic congestive heart failure secondary to either ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 70:359-363

21. Hogenhuis J, Voors AA, Jaarsma T, Hoes AW, Hillege HL, Kragten JA, van Veldhuisen DJ (2007) Anaemia and renal dysfunction are independently associated with BNP and NT-proBNP levels in patients with heart failure. Eur J Heart Fail 9:787-794

22. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 130:461-470

23. Yang RY, Rabinovich GA, Liu FT (2008) Galectins: structure, function and therapeutic potential. Expert Rev Mol Med 10:e17

24. Henderson NC, Mackinnon AC, Farnworth SL, Kipari T, Haslett C, Iredale JP, Liu FT, Hughes J, Sethi T (2008) Galectin-3 expression and secretion links macrophages to the promotion of renal fibrosis. Am J Pathol 172:288-298

25. Smith GL, Lichtman JH, Bracken MB, Shlipak MG, Phillips CO, DiCapua P, Krumholz HM (2006) Renal impairment and outcomes in heart failure: systematic review and meta-analysis. J Am Coll Cardiol 47:1987-1996

26. Hillege HL, Girbes AR, de Kam PJ, Boomsma F, de Zeeuw D, Charlesworth A, Hampton JR, van Veldhuisen DJ (2000) Renal function, neurohormonal activation, and survival in patients with chronic heart failure. Circulation 102:203-210

27. Smilde TD, Damman K, van der Harst P, Navis G, Westenbrink BD, Voors AA, Boomsma F, van Veldhuisen DJ, Hillege HL (2009) Differential associations between renal function and "modifiable" risk factors in patients with chronic heart failure. Clin Res Cardiol 98:121-129

28. Deepa M. Gopal, Maya Kommineni, Nir Ayalon, Christian Koelbl, Rivka Ayalon, Andreia Biolo, Laura M. Dember, Jill Downing, Deborah A. Siwik, Chang-seng Liang and Wilson (2012) Relationship of Plasma Galectin-3 to Renal Function in Patients With Heart Failure: Effects of Clinical Status, Pathophysiology of Heart Failure, and Presence or Absence of Heart Failure. *J Am Heart Assoc* 2012, 1: doi: 10.1161/JAHA.112.000760