Research Article

Utility of Plasma Galectin-3, a Novel Marker of Fibrosis, for Evaluation of Syrian Patients with chronic Heart Failure

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ABSTRACT

Recent studies indicate that galectin-3 is one of the most likely mediators between macrophage activation and myocardial fibrosis, and it plays a role in heart failure development and progression. This study sought to explore the role of galectin-3, a new biomarker in Syrian patients with heart failure. A total of 134 baseline plasma samples were enrolled in this study. Plasma levels of galectin-3, and amino-terminal pro-brain natriuretic peptide (NT-proBNP) were measured in 112 patients with chronic HF, and in 22 patients with non-cardiac dyspnea. The HF patients were classified according to the New York Heart Association (NYHA) classification criteria. Galectin-3 levels were significantly higher in patients with HF, versus non-cardiac dyspneic patients (p=0.0094), and levels of galectin-3were significantly higher in non-cardiac dyspneicpatients, versus controls (p<0.0001). Similarly, levels of NT-proBNP were significantly higher in patients with HF versus non-cardiac dyspneic patients (p<0.0001), and in contrast to galectin-3, NT-proBNP levels were not different in those non-cardiac dyspneic patients versus controls (p=0.11). For HF patients, galectin-3 levels were higher in HF patients versus controls (p<0.0001). Higher galectin-3 levels were associated with measures of heart failure severity, including higher NYHA class and higher levels(r=0.21, p=0.00013). However, galectin-3 did not seem to have similar utility as NT-proBNP for the diagnosis of HF in this study (P = 0.02). Plasma gal-3 levels were elevated in patients with moderate to advanced chronic HF, those levels were correlated with increased severity of HF. galectin-3 could be a useful marker for the diagnosis of non-cardiac dyspneic patients but not for the diagnosis of HF.

Keywords: Chronic heart failure, Diagnosis, Dyspnea, Galectin-3.

INTRODUCTION

Heart failure (HF) is a complex disorder in which a number of physiological systems participate, acting on the myocyte and interstitial cells.[1] and recent studies, both in acute and chronic HF indicate that it is still associated with high morbidity and mortality. Early identification of high-risk patients may favorably affect outcome and biomarkers are increasingly being recognized to have important clinical value in this respect.[2]

Several biomarkers are used for diagnosis and prognosis of HF patients.[3] Brain natriuretic peptide (BNP) and its cleavage equivalent amino-terminal proBNP (NT-proBNP) are established serum markers for diagnosis and prognosis in acute or chronic HF. Both peptides are derived from the cleavage of proBNP, whose production is rapidly up-regulated when cardiomyocytes are stretched. These peptides identify increased wall stress, and have proven utility in confirming the diagnosis of acute HF in breathless subjects and predicting adverse outcomes in these patients. Aside from myocardial stretch, other mechanisms, such as inflammation, might also play a role in HF, whereas these processes might not be reflected by natriuretic peptide levels.[4] A growing body of evidence links macrophage activation and fibrosis to the pathogenesis of HF. Among a number of candidate mediators, galectin-3 is one of the most robustly over-expressed mediators in failing hearts in microarray studies.[5] Galectin-3 is a soluble β-galactoside-binding

lectin that plays an important regulatory role in cardiac fibrosis and remodeling, which are key contributing mechanisms to the development and progression of HF.[2]

Galectin-3 is secreted by activated macrophages and modulates several physiological and pathological processes that contribute to HF,[3] including myofibroblast proliferation, fibrogenesis, tissue repair, cardiac remodeling and inflammation.[6] Galectin-3 is up-regulated in cardiac resident macrophages in hypertrophied rat myocardium, even before the onset of frank HF.[7,8]

The up-regulation of myocardial galectin-3 has initially been demonstrated in a rat model of HF-prone hypertensive hearts. Subsequently, elevated levels of plasma galectin-3 in patients with acute and chronic HF were consistently associated with adverse outcome.[3] These observations have lead to current research directions that focus on the modulation of galectin-3 as a potential diagnostic and therapeutic target.[6]

Therefore, we investigated the value of galectin-3 in HF and dyspneic Syrian subjects, and compared this to a marker for neurohormonal activation (NT-pro-brain natriuretic peptide (NT-proBNP)).

MATERIALS AND METHODS

This study examined 134 plasma samples of dyspneic Syrian patients collected at three regional teaching hospitals; Alassad Hospital, Almouassat Hospital, and Albassel Hospital in Damascus, Syria. Samples were collected during the period between May 5th 2010 to

January 20th 2011. For each patient, a study physician assigned a final diagnosis of HF (from any cause), versus non-cardiac dyspnea using all hospital records pertaining to the patient. These records included office notes, clinical history, echo cardiographic images, and results of all laboratory tests. 112 patients with chronic HF were categorized according to the New York Heart Association (NYHA) functional classification (II-IV). Baseline demographic data, functional status, cardiovascular risk factors and medication were also recorded. Among the 22 dyspneic patients without HF, 10 patients had pneumonia, 5 patients had chronic obstructive pulmonary disease (COPD), and 7 patients had asthma. Control subjects were 26, sex- and age-matched study patients. A written informed consent was obtained from all individuals.

Laboratory measurements

At the time of presentation, 10 mL of blood was drawn from the antecubital vein of all patients and transferred in chilled disposable tubes containing ethylene diamine tetra acetic acid (EDTA). The blood samples were immediately centrifuged at 3,500 rpm for 15 min at 4°C. The plasma was transferred into 1 mL cryotubes, and stored at -80°C for later measurement of gal-3, and NT-proBNP.

Plasma galectin-3 levels were determined using enzyme-linked immunosorbent assay kits (BioVendor, Modrice, Czech Republic) and measured on an ELISA plate reader ABBOTT-tecan-sunrise (ABBOTT, Illinois, USA). Calibration of the assay was performed according to the manufacturer's protocol. Values were normalized to a standard curve. The intra-assay and interassay variances for gal-3 were 6.4% and 11.4%, respectively. And plasma NT-proBNP levels were determined using an enzyme-linked immunosorbent assay kits (Biomedica, Eching, Germany) and measured on a ELISA plate reader ABBOTT-tecan-sunrise (ABBOTT, Illinois, USA). Calibration of the assay was according to the manufacturer's protocol. Values were normalized to a standard curve. The intra-assay and interassay variances for NT-proBNP were 7% and 12%, respectively. Galectin-3 and NT-proBNP measurements were performed at the central blood bank, Damascus, Syria.

Statistical analyses

Statistical analyses were performed using Graph PadPrism® for Windows v6.01 (GraphPad Software, CA, USA). A P fortrend<0.05 was considered statistically significant. Base-lined emographics were given in means ± standard deviation (SD). Pearson's correlation test was used to analyze the association between galectin-3 and NT-proBNP. Receiver-operating characteristic (ROC) curves were utilized to evaluate the utility of gal-3, compared to NT-proBNP for the diagnosis of HF. ROC analyses were performed using MedCalc® v12.4.0 (Med Calc Software, Ostend, Belgium).

RESULTS

Patient characteristics

As previously mentioned, a total of 134 baseline plasma samples from all patients were available and were included in the analysis. 22 (13 males and 9 females) dyspneic patients without HF were enrolled, and a total of 112 (84 males and 28 females) CHF patients were enrolled. For CHF population, the mean age was 57±14 years and left ventricular ejection fraction (LVEF) was recorded by echocardiography: mean LVEF was 38±14, the distribution of NYHA classification In this population was 40, 43, and 29 for class II, class III, and class IV respectively. We divided galectin-3 plasma levels (10.8-25.98 ng/mL) in patients with HF according to NYHA classification (NYHA II into three categories: 10.8- 21.6 ng/mL ; NYHA III: 13.4- 24.5 ng/mL ; NYHA IV: 15.2– 25.98 ng/mL). Galectin-3 levels in dyspneic patients without HF were 12.8- 20.3 ng/mL, and in controls were 9.1- 17.2 ng/mL. And NT-proBNP plasma levels (2.9- 108.2 fmol/mL) in patients with HF, according to NYHA classification were NYHA II: 3.1- 54.2 fmol/mL; NYHA III: 2.9- 68.6 fmol/mL; NYHA IV: 3- 108.2 fmol/mL. In dyspneic patients without HF were 2.7- 22 fmol/mL, and in control patients were 3- 7.02 fmol/mL. Baseline characteristics for HF patients are depicted in Table 1.

**Table 1:** Baseline characteristics for HF patients

| Number of HF Subjects	112
Age, mean (SD), years	57 (14)
Female, N (%)	28 (25%)
NYHA, N (%)	
Class II	40 (36%)
Class III	43 (38%)
Class IV	29 (26%)
LVEF, mean (SD)	38 (14)
BMI, mean (SD), kg/m2	29.4 (7.9)
Hypertension, N (%)	72 (64%)
Smoker, N(%)	19 (17%)
Nt-proBNP, mean (SD), fmol/mL	25.6 (21.7)
Galectin-3, mean (SD), ng/ml	17.4 (2.8)
Medications (%)	
Beta-blocker	67%
Diuretics	96%
Digoxin	22%
Nitrate	14%
Angiotensin-converting enzyme inhibitor (ACE inhibitors)	73%
Angiotensin receptor blocker (ARB)	11%

Galectin-3 in breathless patients

As documented previously, of the 134 patients in this study, 122 (83.6%) had CHF as a final diagnosis, and 22(16.4%) had dyspnea without HF. For this population, concentrations of galectin-3 were higher in the 134 patients versus controls (17.2 ± 2.7 vs. 13.5±2.23 ng/ml, p<0.0001), concentrations of gal-3 were also significantly higher in patients with HF versus non-cardiac dyspneic patients (17.4±2.8 vs. 16.2±1.7 ng/ml, p=0.0094), and concentrations of galectin-3were significantly higher in non-cardiac dyspneic patients versus controls (16.2±1.7 vs.13.5±2.23 ng/ml, p<0.0001). Similarly, concentrations of NT-proBNP were higher in the 134 patients versus controls (22.4±21.3 vs. 3.99±0.9fmol/ml, p<0.0001), concentrations of NT-proBNP were higher in patients with HF versus non-cardiac dyspneic patients (25.6±21.8 vs. 5.96±5.5fmol/ml, p<0.0001). In contast to galectin-3, concentrations of NT-proBNPwere not different in those non-cardiac dyspneic patients versus controls (5.96±5.5 vs. 3.99±0.9fmol/ml, p=0.11). These values are displayed in Figure 1.

Plasma biomarkers in patients with HF

As can be seen in Figure 2, Galectin-3 levels were higher in HF patients versus controls (17.4 ±2.8 vs. 13.5±2.23 ng/ml, p<0.0001). Galectin-3 correlated with severity of heart failure as indicated by NYHA Class, Patients in NYHA class II, III, and IV had higher galectin-3 levels versus controls (P<0.0001). Patients in NYHA class III had higher galectin-3 levels versus those in NYHA class II (p=0.01), Patients in NYHA class IV had higher galectin-3 levels versus those in NYHA class III (p=0.03). Patients in NYHA class IV had higher galectin-3 levels versus those in NYHA class II (p<0.0001). NT-proBNP levels were higher in HF patients versus controls (25.6 ±21.8 vs. 4±0.9fmol/ml, p<0.0001). Patients in NYHA class II, III, and IV had higher NT-proBNP levels versus controls (P<0.0001). patients in NYHA class III had higher NT-proBNP levels versus those in NYHA class II (p=0.02), Patients in NYHA class IV had higher NT-proBNP levels versus those in NYHA class III (p=0.03), and also Patients in NYHA class IV had higher NT-proBNP levels versus those in NYHA class II (p=0.0006).
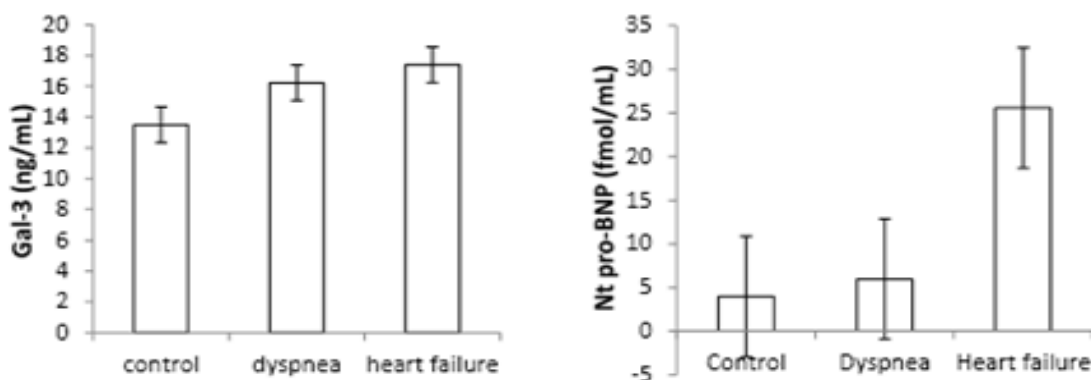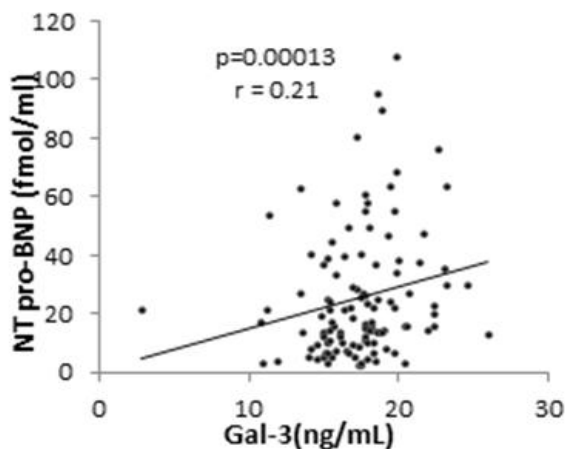

Figure 1: Plasma levels of galectin-3 and NT-proBNP levels in HF patients (n=112), non-cardiac dyspnea patients (n=22), and healthy controls (n=26). Median and range of values for galectin-3: [controls 13.5 (9.1-17.2 ng/mL), HF 17.4 (10.8-25.98 ng/mL), and dyspnea 16.2 (12.8-20.3 ng/mL)].median and range of values for NT-proBNP: [controls 3.99 (3- 7.02 fmol/mL),HF 25.6 (2.9- 108.2 fmol/mL), and dyspnea 5.96 (2.7- 22 fmol/mL)].
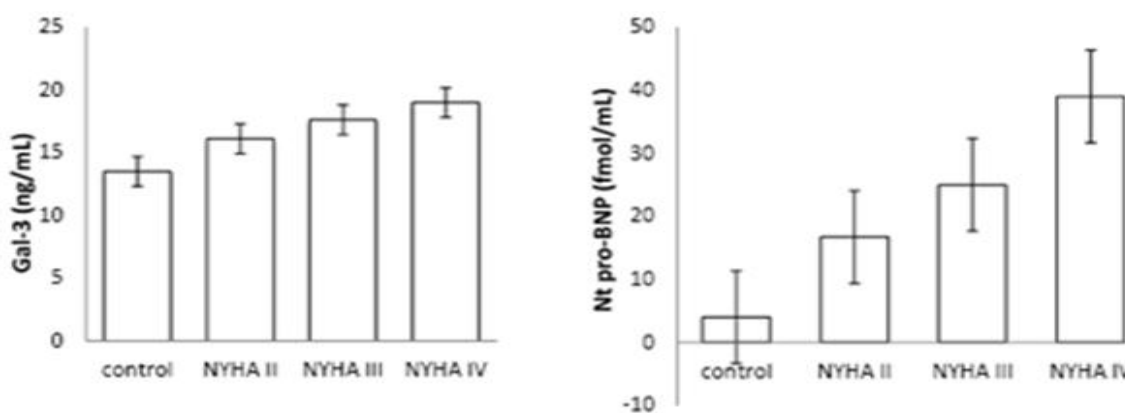
Figure 2: Plasma levels of galectin-3 and NT-proBNP in HF patients (n=112) and healthy controls (n=26) in relation to clinical disease severity as assessed by NYHA classification. Median and range of values for galectin-3: [controls13.5 (9.1-17.2ng/mL), NYHAII 16.09 (10.8- 21.6ng/mL), NYHAIII17.59 (13.4- 24.5ng/mL), NYHAIV18.98(15.2– 25.98 ng/mL)]. Median and range of values for NT-proBNP: [controls3.99 (3- 7.02fmol/mL), NYHAII 16.7 (3.1- 54.2fmol/mL), NYHAIII24.96 (2.9-68.6fmol/mL), NYHAIV38.96 (3- 108.2fmol/mL)].

Association of Galectin-3 with NT-pro BNP

As illustrated in Fig.3, galectin-3 levels were found to be higher when NT-proBNP levels were also higher. There was a modest correlation between NT-proBNP levels and galectin-3 levels (r=0.21, p=0.00013).

Figure 3: Relationship between plasma Galectin-3 levels and NT-proBNP levels.

The diagnostic potency of galectin-3

Receiver-operating characteristic (ROC) curves were utilized to evaluate the utility of galectin-3 compared to NT-proBNP for the diagnosis of HF patients. Here we designed combined receiver-operating characteristic (ROC) curves with both base-line NT proBNPand base-line galectin-3 for diagnosis of HF patients. ROC curves are shown in Fig. 4. As can be seen in table 2, ROC analysis examining NT-proBNP for diagnosis of HF yielded an area under the curve (AUC) of 0.94 (P<0.0001) for NT-proBNP. The ROC analysis for galectin-3 for the diagnosis of HF showed an AUC of 0.86 (P<0.0001). Table 3 presents the comparison of the two ROC curves; the NTproBNP had significantly greater AUC than gal-3 for the diagnosis of HF (P = 0.0219).
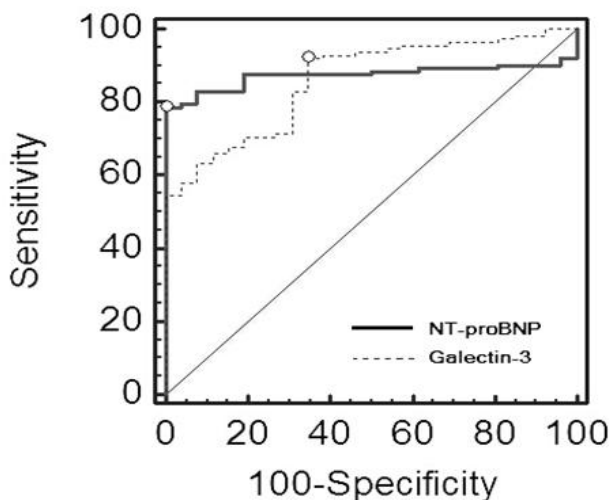
Figure 4: Combined receiver-operating characteristic (ROC) curves for base-lineNT-proBNPandgalectin-3for the diagnosis of HF patients.

Table 2: ROC curve data

	AUC[a]	SE[b]	95% CI[d]
NtProPNB	0.941	0.0200	0.888 to 0.974
Galectin-3	0.860	0.0353	0.790 to 0.913

a : Area under the ROC curve (AUC); b : Standard Error; d : Confidence Interval

Table 3: Pair wise comparison of ROC curves

NtProBNP ~ Galectin-3	
Difference between areas	0.0816
Standard Error	0.0356
95% Confidence Interval	0.0118 to 0.151
Significance level	P = 0.0219

DISCUSSION

We have shown that plasma galectin-3 levels were elevated in Patients with moderate to advanced chronic HF. Although increased levels of galectin-3 were associated with increasing severity of HF as assessed by increasing levels of NT-pro BNP, galectin-3 remained an independent marker for HF. We found that galectin-3 could be a useful marker for the diagnosis of dyspneic patients without HF because of its inflammatory role. In contrast, Galectin-3 with suggested utility in HF evaluation did not seem to have similar utility as NT-proBNP for the diagnosis of HF in our study.

After an initial insult to the myocardium, cardiac remodeling occurs as a compensatory mechanism, and this will ultimately lead to left ventricular dysfunction and HF. This complex process with an increase of left ventricular wall thickness and dilatation and reshaping of the left ventricle leads to lengthening or hypertrophy of cardiomyocytes with insufficient angiogenesis leading to metabolic and ischemic problems.[2] Because cardiomyocytes represent only one-third of the myocardium, it is not surprising that cells other than myocytes are also involved in the progression of HF. It is also well known that in the failing human heart, immunologic and inflammatory processes play an important role. Pro inflammatory cytokines modulating several cardiovascular mechanisms and activated monocytes and macrophages are all involved in the pathogenesis of not only ischemic, but all forms of HF.[4] Activation and proliferation of fibroblasts, which increases the synthesis of fibrillar collagen and activates the inflammatory response, plays an important role in this proces. Galectin-3 is likely to play a role in this process, and has been shown to interact with various ligands located at the extracellular matrix.[2] Galectin-3 is a β-galactoside-binding animal lectin and is highly expressed and secreted by macrophages. Galectin-3 is involved in fibrosis in various organs including the lung, liver, kidney, and heart,[5] and it promotes macrophage migration, fibroblast proliferation and collagen synthesis, or the development of fibrosis.[2] Cardiac macrophages are activated at an early stage in failure-prone, hypertrophied

hearts and these macrophages express galectin-3. Unlike galectin-3, common biomarkers used in the diagnosis of cardiovascular disease such as the natriuretic peptides, cardiac troponins, and c-reactive protein are generally elevated as a result of the disease process and do not substantively contribute to disease progression.[9]

A role for galectin-3 in the progression of HF first came to attention in a sequence of basic science and animal studies performed by sharmaet al.[7] First identified by a DNA micro-array, the galectin-3 gene was the most prominently over-expressed gene in HF rat myocardium, with a greater than five-fold rise compared with compensated hypertrophied myocardium. Second, the protein was also found in the highest quantities in rat hearts with the highest degree of cardiac fibrosis. These rats also went on to develop HF. And galectin-3 was found to be co-localized with infiltrating macrophages within the myocardium. normal rats had neither galectin-3 nor infiltrating macrophages. These findings were confirmed in human heart myocardial biopsies, with higher levels of galectin-3 in those with poorer LVEFs.[9] Astudy by Liu Y et al.[10]found that intra pericardial infusion of galectin-3 for 4 weeks in adult male rats led to 1. enhanced macrophage and mast cell infiltration, increased cardiac interstitial and perivascular fibrosis, and cardiac hypertrophy, 2. Increased transforming growth factor-β (TGF-β) expression, and 3. decreased cardiac performance, as for example shown by systolic and diastolic cardiac performance.[10] In an animal model of renal fibrosis, Henderson N et al.[11] Found that disruption of the galectin-3 gene by a knockout galectin-3 gene does not affect macrophage recruitment or macrophage pro inflammatory cytokine profiles, but decreases renal fibrosis. Adoptive transfer of wild-type macrophages restored the fibrotic phenotype in galectin-3 knockout mice. This study demonstrated that galectin-3 expression and secretion is a key mechanism linking macrophages to myofibroblast activation and tissue fibrosis.[11] Here in our study, we translate these experimental findings into a clinical application, we have showed that gal-3 levels are elevated in a discriminatory fashion in those with HF as compared to normal controls, with increasing levels according to disease severity, including higher New York Heart Association class, and thus confirming some previous reports of DeBoeret al.[3], Felkeretal.[12] Uelandetal.[1], Christenson et al.[6], Lok et al.[2], Gullestad et al.[13], and Shao et al.[14]

Lee et al.[15] have already proposed a possible multimarker strategy and classified serum biomarkers in HF into four categories: neurohormonal markers, markers of myocyte injury, markers of matrix remodeling, and inflammation-related markers.[4] The natriuretic peptides are neurohormonal markers. They have emerged as the most well validated biomarkers in HF and are recommended by current guidelines for both establishing diagnosis and risk stratification of patients with chronic heart failure.[12] In the current study, plasma NT-proBNP levels are significantly elevated in patients with HF as compared to

normal controls, with increasing levels according to disease severity. these finding are similar to previous data of deFilippi et al.[16] and Schou et al.[17] Natriuretic peptides are 'loading markers', which readily and strongly respond to ventricular stress, while galectin-3 levels are viewed as a marker of interstitial fibrosis, less responsive to (un-)loading. In support of this theory, Milting et al.[18] showed that unloading of poorly contractile hearts with assist devices causes a robust decrease in various neurohormones, including natriuretic peptides, but not galectin-3.[3] Therefore, The value of galectin-3 levels is independent from (NT-pro-BNP) levels. Our data confirms and extends previous data on association between galectin-3 levels and NT-pro-BNP levels. We found a significant positive correlation between galectin-3 and NT-proBNP, these data agreed with results of other studies of deFilippietal.[16] Felker et al.[12] Ueland etal.[1]Lok et al.[2] Gullestad et al.[13] and Shah et al.[8] Also, our data have showed that NT-proBNP was able to identify those HF patients better than Galectin-3. Our finding is in agreement with the study ofvan Kimmenade et al.[4]

We furthermore evaluated if galectin-3 would be elevated in dyspenic patients without HF, since it is well described that galectin-3 plays a central role in the inflammatory response.[3]Galectin-3 has been found to induce fibrosis, at least partly through direct interaction with fibroblasts. It also appears to be involved in transition from acute inflammation to a state of chronic and non-resolving inflammation.[1] In a previous report by Ueland et al,[1] it was found that patients with increased galectin-3 levels also had increased levels of CRP and high total leukocyte counts, suggesting a link between galectin-3 and inflammation. de Boer et al.[3] also observed a significant trend that with increasing galectin-3 levels pro-inflammatory cytokines (vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), C-reactive protein (CRP), and transforming growth factor-β1 (TGF-β1)) levels also rise. These data support the observation that galectin-3 may be involved in inflammation, as well as in HF.Galectin-3 regulates immune response and inflammation. In general, it is a powerful pro-inflammatory signal. Certain cells produce and secrete large amounts of galectin-3 as a response to various inflammatory stimuli. When secreted or externalized, galectin-3 may affect inflammatory cells by an autocrine or paracrine mechanism; it triggers/promotes respiratory burst in neutrophils and monocytes and induces mediators release by mast cells. It promotes adhesion of human neutrophils to laminin and endothelial cells, and acts as a chemoattractant for monocytes and macrophages. Galectin-3 can also bind LPSs of Klebsiella pneumoniae, and it was also shown to interact with glycolipid component of Mycobacterium tuberculosis and to accumulate in pathogen-containing phagosomes in macrophages during the course of the infection.[19] In our study, a significant trend was observed with increasing galectin-3 levels in non-cardiac dyspenic patients as compared to normal controls. In contrast, NT-proBNP levels were not different in non-cardiac dyspneic patients

versus controls. This might be due to NT-proBNP being a loading marker that increases as a response to ventricular wall stress, whereas inflammation processes might not be reflected by natriuretic peptide levels. We have also found that plasma galectin-3 and NT-proBNP levels are significantly elevated in dyspenic patients with HF as compared to dyspenic patients without HF. this finding is in agreement with both van Kimmenade et al[4] and Shah et al.[8]

CONCLUSION

Galectin-3 is a protein that has been directly implicated in the fibrosis in multiple organ systems, including the heart. There is ample evidence that galectin-3 is directly involved in the pathophysiology of cardiac injury and progression to HF, making it a potential target for future therapy.

In the current study, we concluded that plasma gal-3 levels are elevated in HF patients. These levels were associated with measures of heart failure severity, including NYHA class and NT-proBNP levels. Galectin-3 may be a useful marker for the diagnosis of non-cardiac dyspneic patients. However, NT-proBNP was superior to galectin-2 for dignosing HF.

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