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Additional value of Galectin-3 respect to BNP in acute heart failure patients with preserved ejection fraction

Abstract

Background: Almost half of patients with acute heart failure (AHF) experienced a Heart failure with preserved ejection fraction (HFpEF). HFpEF diagnosis is difficult using traditional diagnostic tools; specific laboratory markers able to recognize this setting are lacking. Galectin-3 (Gal-3) is an emerging biomarker useful in individuals at risk for HF, in both acute and chronic HF.
 The aim of our study is to analyse the relation and prognostic value of Gal-3, BNP and renal dysfunction in patients with HFpEF compared to patients with Heart Failure and reduced ejection fraction (HFrEF). We also evaluated the association between Gal-3 and the degree of diastolic dysfunction, LV geometry and stiffness.

Methods: We enrolled 98 patients with AHF and we measured Gal-3, BNP, C-reactive protein (CRP) and estimated glomerular filtration rate (eGFR) within 12 hours since hospital admission. On the basis of echocardiographic findings we divided our sample into two groups: patients with HFrHF (ejection fraction<50%) or HFpEF (ejection fraction>50%).

Results: No differences in Gal-3 levels were found in the two subgroups (HFrEF; 19,5±5,1 ng/mL; HFpEF; 20,5±8,7 p=0,56). Gal-3 was inversely related to renal dysfunction (Gal-3 vs eGFR r=-0,30, p=0,01) but did not correlate with BNP levels (r=0,22, p=0,06). Higher levels of Gal-3 were associated with more advanced diastolic dysfunction only in HFpEF (Impaired relaxation pattern: Gal-3 =15,2±4,3 ng/mL; pseudo-normal filling pattern: Gal-3 =18.7±7.1 ng/mL and restrictive filling pattern: Gal-3 =27,5±10,1 ng/mL p=0,009). In addition Gal-3 was related to diastolic LV stiffness (all patients; r=0,4 p=0,01 HFpEF r=0,58 p<0,001). Cox regression analysis showed that Gal-3 was related to poor outcome independently from renal dysfunction and other risk factors both in HFpEF patients (HR: 1.22 [1.08-1.38]; p=0,001) both in HFrEF patients (HR: 1.15[1.04-1.27]; p=0,007).

Conclusions: Gal-3 is not able to discern HFrEF respect to HFpEF patients. Unlike BNP, it is related to diastolic dysfunction severity and LV stiffness in HFpEF. Gal-3 demonstrates a prognostic role independently from renal dysfunction in subjects with HFpEF.

Introduction

The great variability that characterizes Heart Failure (HF) syndrome depends on the heterogeneity of patient population and multiple etiologies. Around half patients with acute heart failure (AHF) experienced an Heart failure with preserved systolic function (HFpEF) with left ventricular ejection fraction (LVEF) more than 50%. Unfortunately, most interventional Trials focused on patients with Heart failure and reduced ejection fraction (HFrEF), and poor data are available for patients with HFpEF. These patients are more often old, female, obese with higher incidence of hypertension and atrial fibrillation (AF). [1] In this context additional parameters for both diagnostic and prognostic assessment may facilitate HF diagnosis identifying the pathophysiological process responsible for the disease as well as prognostic assessment. [2] Early diagnosis is mandatory for both therapy optimization and risk categorization. In this scenario the role of some specific biomarkers together with imaging evaluation appears a priority (prioritary non esiste) for the assessment of patients with acute HF. For this reason, there is an increasing interest in the development of new biomarkers and a great number of laboratory tests have recently been studied. [3] Galectin-3 (Gal-3) is an emerging biomarker useful in individuals at risk for HF, in both acute and chronic HF, in HF management and finally for prognostic purposes. [4,5] A recent pooled-meta-analysis in patients with AHF showed that circulating Gal-3 plasma levels are a stronger predictor of outcome in terms of death and HF re-hospitalization.[6]

Gal-3 is a beta galactoside-binding lectin secreted by macrophages. In response of this activation, galectin-3 initiates a pro-fibrotic program: other macrophages, pericytes, and myofibroblasts are consequentially activated. At the end of the cascade the fibroblasts lead to the deposition of collagen into the extracellular matrix. This process leads to a progressive cardiac fibrosis and consequent pathological remodeling of the myocardium structure.[7] Myocardial fibrosis and extracellular matrix collagen deposition are two major players in HFpEF pathophysiology. [2] However the diagnostic evaluation of HFpEF remains underestimated due to the absence of a universally accepted definition of the disease and consequent diagnostic inaccuracy. Moreover, several studies evaluated Gal-3 in chronic HF patients and mainly in subjects with systolic dysfunction. [8,9] Thus, less data are available on subjects affected to HFpEF and its diagnostic and prognostic role remains debated. Finally, increased levels of Gal-3 have been recently found in

patients with renal dysfunction and chronic kidney disease (CKD), and this condition appears strictly related to protein levels. [10] Based on these concerns, the aim of this study was to investigate the diagnostic role, of Gal-3 and B-type natriuretic peptide (BNP) in patients with HFrEF compared to patients with HFpEF. We also analysed the prognostic value of two biomarkers and the relation between Gal-3 and renal dysfunction.

Methods

Population

We evaluated 98 patients with AHF admitted to our Department since September 2012 to February 2015. Five patients were subsequently excluded, lacking echocardiographic data. We performed echocardiography and we measured Gal-3 and BNP within 12 hours since hospital admission. Patients were enrolled within 12 hours of hospital admission with diagnosis of new onset or exacerbated AHF, with signs and symptoms of acute decompensated HF: dyspnoea, orthopnoea, pulmonary rales, third tone, peripheral oedema or exercise intolerance, chest X-ray signs of pulmonary pressure overload, dilated jugular veins, hepathic-jugular reflux. We assessed the congestion score index by five principal signs giving for each sign 1 point in a scale ranging from 1 to 5. We considered the following clinical signs: third heart sound, pulmonary rales, jugular venous stasis, hepatomegaly, and peripheral edema. All these patients were screened for Diur HF trial (NCT).

We previously excluded patients with poor acoustic window on echocardiography, respiratory dyspnoea due to bronchial asthma, interstitial lung disease (ILD) or chronic obstructive pulmonary disease (COPD). We excluded patients with acute or past myocardial ischemia ($\geq$6 months) by anamnestic investigation, serial electrocardiographic (ECG) and troponin and myoglobin measurement. We also excluded patients with severe renal insufficiency in haemodialysis and renal replacement treatment, neoplastic, hematologic and immune diseases with systemic involvement. The echocardiography was performed using HP Sonos 5500 machine. All the exams were performed by two experienced cardiologists according to the instructions provided by the American Society of Echocardiography. [11] Therefore principal measurements were recorded and independently reviewed by two distinct physicians. We obtained the two-dimensional image through the parasternal long and short axis views and apical four and two chamber views. Standard echocardiographic M-mode measurements were used for the determination of left ventricle (LV) diameters and wall dimensions. Atrial and ventricular areas were calculated tracing endocardial borders including the papillary muscles. The systolic and diastolic volumes and ejection fraction were determined using apical two-and four chamber views by Simpson biplane formula. We

evaluated three consecutive cardiac cycles to obtain average pulsed Doppler transmitral flow velocity during early diastole velocity (E wave) and late diastole velocity (A wave) ratio (E/A) and the deceleration time (DT) of E. The isovolumetric relaxation time (IVRT) was obtained in the apical five chambers view placing the pulsed Doppler between the LV outflow tract and the mitral inflow tract. The IVRT was measured in milliseconds between the end-ejection time and the beginning of ventricular filling time.

Placing the cursor laterally and medially to the mitral annulus we estimated mitral annulus movement by apical four-chamber Tissue Doppler Imaging (TDI). We obtained the recordings of systolic peak velocity (S'), early diastolic myocardial velocity (e') and atrial systole velocity (A'), for three consecutive cardiac cycles. We calculated the ratio of peak early diastolic filling velocity and septal TDI early diastolic velocity (E/e'). We estimated the systolic pulmonary artery pressure (PAPs) by continuous Doppler at tricuspid valve level. The Tricuspidal Anular Plane Systolic Excursion (TAPSE) was obtained by placing the M-mode cursor laterally to the tricuspid annulus; it reflects the systole right ventricle shortening apex-base and it is an index of right ventricle (RV) systolic function. Each result was normalized for body surface area (in square meters), calculated by the weight and height of each patient.

We assessed the diastolic pattern by the following cut-off: impaired relaxation pattern [diastolic dysfunction type I (E/A <0.8, DT > 200 msec, IVRT> 100 msec, E/e'<8 septal and lateral)]; pseudo-normal filling pattern [diastolic dysfunction type II (E/A 0.8-1.5 and decreases by 50% during the Valsalva maneuver, DT 160-200 msec, IVRT <90 msec, 9>E/e'>12)]; restrictive filling pattern [diastolic dysfunction type III (E/A>2, DT <160 msec, IVRT <60 msec and average E/e' ratio >13)]. The ratio of peak early diastolic filling velocity and septal TDI early diastolic velocity (E/e') were assessed. Normal LV filling pressure when the ratio E/e' ≤ 8; a grey zone (where additional parameters are necessary) when the ratio is 8<E/e'≤ 15 and finally high filling pressure when E/e'>15. (12) Relative wall thickness (RWT) was calculated by the formula (2 x posterior wall thickness)/(LV internal diameter at end-diastole). RWT is an expression of an increase in LV mass as either concentric (RWT > 0.42) or eccentric (RWT< 0.42) hypertrophy and allows the presence of concentric remodeling (normal LV mass with increased RWT). LV mass is calculated by CUBE formula. [112]

Diastolic stiffness was assessed by the ratio E/e' (index of LV filling pressure) and left ventricular diastolic diameter (LVEDD) (index of left ventricular volume). The ratio represents diastolic stiffness and this was measured for each subject in the study. [13] We established a cut off>1.8 as suggestive for increased LV stiffness. On the basis of echocardiographic findings we dived our sample in two groups: patients with HFrHF (EF<50%) or HFpEF (EF>50%).

RV parameters indicative of abnormal right ventricular function where TAPSE ≤ 18 mm; and elevated pulmonary pressure when PAPs ≥ 40 mmHg. Renal dysfunction is defined by a cut off value of eGFR≤60 ml/min/1,73 m^2. [14]

Ethics Statement: This study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by our hospital's Institutional Review Board of Siena (South East Wide Area Ethic Committee of Tuscany region Italy) and all patients gave their signed informed consent.

Endpoints- 1- To evaluate levels of Gal-3, BNP, C- reactive protein (CRP) and renal dysfunction prevalence [in terms of creatinine and estimated glomerular filtration rate (eGFR)] in group with HFrEF and HFpEF 2) To study the relation between Gal-3 and BNP with the degree of diastolic dysfunction, LV geometry and stiffness, 3) to analyse the prognostic impact of Gal-3 in terms of death and re-hospitalization for cardiovascular causes during 6 months follow up.

Follow-up- Patients were followed for six months after discharge with clinical examinations or telephone calls. Composite outcome were considered the sum of death and re-hospitalization for cardiovascular causes . We considered for analytical purposes as hospitalization for HF events such as acute decompensate HF, acute coronary syndromes complicated by HF, ventricular arrhythmias associated with LV dysfunction, or HF associated with worsening renal function.

Laboratory assay

All blood samples obtained from patients were collected with a sterile disposable syringe containing EDTA. Samples were analysed within using the Alere Triage BNP Test. This test is an immunoassay in a single-use plastic cartridge fluorescently labeled monoclonal antibody against BNP labeled with a fluorescent dye and BNP. Plasma BNP was measured with Triage BNP Test (Biosite Inc., San Diego, CA, USA).

The human Galectin 3 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human Galectin 3 (eBioscience, San Diego, USA). The assay was performed by the measurement of the protein in EDTA plasma. Aliquots of serum samples were stored at temperature ranging from 2° to 8 and the human Galectin 3 level determinated after 24 h. Measurement of Creatinine was done by Jaffé reaction, in which creatinine is treated with an alkaline picrate

solution to yield a red complex. The velocity of color makeup is directly proportional to creatinine concentration measured photometrically. (CREJ2 cobas, Roche system). eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) equation. [15]

Statistical analysis

Continuous variables are expressed as means (± standard deviation), while discrete variables are presented as percentages (%). Patients' laboratory parameters were compared using t-test or one way ANOVA for independent sub-groups, discrete variables were compared with Cochran-Mantel-Haenzsel and X_2 test. Analysis of variance was done by Levene's test, and if it was breached Welch's correction was used. Pearson's correlation coefficient was used to assess relation among biomarkers. Receiving Operating Characteristic (ROC) curve was performed to assess the relationship among BNP, Gal-3 and creatinine with outcome. Cox regression analysis was used to assess the independent and the dependent relationship among BNP, Gal-3, LVEF and creatinine with death or re-hospitalization at six months. Multivariable analysis was adjusted for age, sex, cardiovascular risk factors as hypertension, diabetes, dyslipidemia and smoke, CKD, LV stiffness, New York Heart Association (NYHA) class. All reported probability values were two-tailed, and a p value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using the SPSS 20.0 statistical software package (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics of enrolled patients- 45 patients with HFpEF (46%) and 53 with HFrEF (54%) were enrolled. Gal-3 levels were 20±7 ng/mL in all patients; 19,5±5,1 ng/mL in patients with HFrEF and 20,5±8,7 in HFpEF (p=0,56). Median BNP levels in all population were 1054±982 pg/ml. BNP was significantly increased in patients with HFrEF compared to HFpEF (1282±694 vs 783±570 pg/ml p=0.03). As we expected patients with HFpEF were more frequently older, female, with history of hypertension and significantly higher BMI. The other baseline characteristics are shown in Table1.

Gal-3 did not correlate with BNP levels in all patients (r=0,22, p=0,06). The relationship wasn't significant also dividing our sample on the basis of EF (HFpEF: r=0,31, p=0,08; HFrEF: r=0,26, p=0,11). Gal-3 correlated with CPR in all patients and also in the two subgroups (all patients: r=0,68, p<0,001; HFpEF: r=0,70, p<0,001; HFrEF r=0.65, p<0,001). (Figure 1) The relationship between Gal-3 and renal dysfunction evidenced an inverse relationship in all patients (Gal-3 vs

eGFR r=-0,30, p=0,01) and in the subgroups with HFpEF (Gal-3 vs eGFR r=-0,40, p=0,02); but no association was found in patients with HFrEF (Gal-3 vs eGFR r=-0.16, p=0,33). (Figure 2)

Gal-3 and echocardiographic data- Echocardiographic data at hospital admission are shown in table 2. Gal-3 levels were not significantly associated with echocardiographic data of LV systolic function and structure (in all patients Gal-3 vs LVEF: r=0,018, p=0.7). Among patients with HFpEF Gal-3 was directly correlated to E/A (r=0,35, p=0,04) and E/e' (r=0,51, p=0,002). Higher levels of Gal-3 were associated with advanced diastolic dysfunction only in the subgroups with HFpEF (Impaired relaxation pattern: Gal-3 =15,2±4,3 ng/mL; pseudo-normal filling pattern: Gal-3 =18.7±7.1 ng/mL and restrictive filling pattern: Gal-3 =27,5±10,1 ng/mL p=0,009) (Figure 3A and 3B). In addition Gal-3 was in relation also with diastolic LV stiffness in all patients and in the subgroups with HFpEF (all patients; r= 0,4 p=0,01 HFpEF r=0,58 p<0,001). (Figure 4) Gal-3 was positively correlated with RWT only in patients with HFpEF (r= 0,47 p=0,01). Although we did not find a difference regarding LV mass in the 2 groups we revealed a trend between Gal-3 and increased cardiac mass in all population (r= 0,36 p=0,02). Finally, Gal-3 did not correlate with indexes of RV function and pressure (Gal-3 vs PAPs r = -0,19, p=0,11, and Gal-3 vs TAPSE r = -0.02, p=0.88).

Association between Gal- 3 and cumulative endpoints- ROC curve analysis was performed to assess the predictive power of Gal-3, BNP and creatinine in relation to outcome. Gal-3, BNP and creatinine were significantly related to poor outcome (BNP: AUC 0,67[0,51-0,82], p=0,02; Gal-3: AUC 0,90[0,83-0,97], p<0,001; Creatinine: AUC 0,76[0,64-0,89], p<0,001). (Figure 5) A cut off value of Gal-3 levels ≥19,5 ng/ml was associated with worse prognosis (sensitivity of 82%, specificity of 83% and accuracy of 81%); in the same way, BNP values more than 400 pg/mL were able to detect 180 days death or re-hospitalization for cardiac causes (sensitivity of 78%, specificity of 85% and accuracy of 82%). Univariate and multivariable analysis showed that Gal-3 was related to adverse outcome independently from other risk factors and renal dysfunction in patients affected to HFpEF (HR: 1,32[1,13-1,53]; p=0,001, univariate; HR: 1.22 [1.08-1.38]; p=0,001, multivariable). (Table 3)

Discussion

Gal-3 shows a pro-fibrotic activity and it is secreted from monocytes/macrophages and epithelial cells; it also has a role in activating various types of cells such as mast cells, neutrophils, lymphocytes as well as monocytes/macrophages. [16] Previous reports did not demonstrate a

precise correlation between Gal-3 and LV structure and function. [17] However high Gal-3 level appears related to unfavorable outcome in several studies, and recent American Heart Association Guidelines have inserted it as additive biomarker for risk stratification.[18] To date, the exact role of Gal-3 in the detection of cardiac dysfunction and its diagnostic role in recognizing HFrEF respect to HFpEF is not clear.

Our data showed that Gal-3 is not able to distinguish between reduced or preserved HF; however, it appears related to diastolic dysfunction severity, LV mass and stiffness. The current findings are much more significant in patients with preserved LVEF respect to the total population. A clear relation was found between Gal-3 and LV stiffness in all patients and particularly in HFpEF. Moreover, Gal-3 increases in relation to CRP values and renal dysfunction, but in cox regression analysis it demonstrated an additive prognostic values respect to eGFR and BNP. Additionally, despite the known relation between CKD and outcome in HF, Gal-3 keeps a prognostic role independently from renal dysfunction in subject with HFpEF. To the best of our knowledge for the first time, our data confirm a specific role of Gal-3 in the detection of HFpEF patients and particularly in those with increased filling pressure and stiffness. [19] Despite we did not directly evaluate the fibrotic amount at tissue level, could potentially means an indirect measure of myocardial fibrosis.

 Differently, our study did not confirm a specific prognostic role of admission BNP values. Indeed, recent reports highlighted the importance of BNP lowering and absolute value at discharge as best prognostic index for outcome prediction. [20,21]

HFpEF has a different pathophysiological mechanism and phenotype with several underling conditions such has hypertension, diabetes, metabolic syndrome and renal dysfunction. All these conditions lead to cardiac and vascular collagen deposition, myocardial fibrosis and hypertrophy, inflammatory activation, endothelial dysfunction with increased oxidative stress.[20][22]

Even though we cannot demonstrate a direct relation with myocardial fibrosis amount, we can likely contemplate that Gal-3 is a specific biomarker of reduced cardiac compliance and a sensible mirror of collagen deposition in cardiac tissue. In clinical practice, it is difficult to precisely quantify cardiac fibrosis but experimental studies in animal models, demonstrated a strict relation among cardiac fibrosis, LV hypertrophy and Gal-3.[23] Indeed, fibrosis production and extracellular matrix deposition are the principal causal players of wall rigidity and chamber stiffness and they appear linked to increased risk of HF development and cardiovascular mortality. [24]Similar results were previously found in the PRIDE study although Authors divided their patients with LVEF cutoff value of 40 % : higher levels of Gal-3 were associated with higher LV filling pressure and more advanced diastolic dysfunction. Gal-3 levels were also related with RV

systolic longitudinal function and RV systolic pressure. However, the current report included all patients with reduced or preserved systolic function, and it did not distinguish HFrEF respect to HFpEF. [177]

Recently the role of Gal-3 has been analyzed in two different trials: in the Aldo DHF study in which patients were randomized to receive spironolactone or placebo, Gal-3 revealed an inverse correlation with exercise tolerance peak VO2 and NYHA class, and it was associated to adverse outcome. [25] Oppositely, in the Relax substudy Gal-3 was not associated to congestion, cardiac remodeling and function, and it is only related to the age and impaired renal function. [26] These data confirmed previous findings showing that prognostic power of Gal-3 is attenuated when it is adjusted for renal dysfunction. [27] Indeed Gal-3 is a recognized fibrotic factor in vascular and kidney districts. It is not surprising that Gal-3 levels are associated with more advanced age, and renal insufficiency; suggesting that Gal-3 is linked to the progression of renal impairment mediated by inflammation , apoptosis, and fibrosis activation. [28]

The renal contribution of Gal-3 levels has been supported by two observations in patients submitted to biventricular pacemaker implantation in which Gal-3 levels at coronary sinus site were lower than blood systemic levels. [29] Therefore in the myocardial tissue of subjects submitted to ventricular assist device, Gal-3 was poorly related to blood sample levels. [30] Both studies raise the questions whether Gal-3 might be due to renal dysfunction and its predictive role may be more related to renal impairment than HF status. Nevertheless, both studies analyzed patients with severe systolic dysfunction, and patients with preserved function were not included, in this sense they do not appear contradictory respect to our observation.

In addition to previous findings, Sharma et al underlined the importance of Gal-3 up-regulation in patients with hypertensive HF and aortic stenosis, suggesting that Gal-3 is not only a marker of myocardial fibrosis and stiffness but it is also causally implicated in HF development and progression. [31]

In accordance with our observation, two large studies have recently showed a strong relation between Gal-3 and Hypertension in a community-based cohort. [24,32]

We could speculate that Gal-3 reflects most of these processes and it may predict HFpEF development in patients with the current comorbidities. [33] Indeed, the current findings demonstrated that although admission BNP was increased in HFrEF, it has not a prognostic value in our population. Different results were found in a larger study by van Kimmenade et al.: the combination of Gal-3 with NT-proBNP was the best predictor for prognosis in subjects with acute HF, however in their study authors did not differ patients on the basis of LVEF. [34] Finally the

prognostic role of Gal3 in HFpEF has been confirmed by Carrasco-Sanchez et al study, in which Gal 3 increased 1.5 fold the risk of all cause mortality and hospitalization during 1 year follow-up period. [35]

Similarly, in our sample, and in particular in patients with HFpEF, Gal-3 increase was related to poor outcome independently from renal dysfunction. It could imply that plasma Gal-3 is influenced by renal Gal-3 production and fibrosis only in subject with reduced systolic function.

Our results need to be confirmed in a larger sample size population screened by detailed echocardiographic measures and cutoff suggestive for diastolic function impairment and high filling pressure.

Limitations

The principal limitation of our study is the relatively small sample size which might have reduced the ability to differentiate some findings in the two groups (HFpEF vs HFrEF). However, our data are well screened with a standardized protocol and precise cutoff regarding LVEF and diastolic measurement, following echocardiographic Recommendation Guidelines for the diagnosis of HF with preserved systolic function. We did not compare diastolic filling measurement with invasive analysis and we cannot ascertain our non invasive results with direct LV pressure/volume study. Blood measurement of biomarkers was obtained only at admission and we do not have seriate measurement during hospitalization and in the follow-up, so we cannot establish which is the best Gal-3 cutoff and significance during time course. Finally, this is an observational retrospective study with several unmeasured confounding factors and comorbidities, and the results should be unscrambled following these conditions

Conclusions

Gal-3 appears to be a promising biomarker in patients with AHF and preserved systolic function. It correlates with diastolic dysfunction severity and LV stiffness, as well as with inflammation and renal dysfunction. The current findings support the relation among Gal-3 and cardiac and vascular fibrosis documented in experimental studies. If confirmed in larger sample sizes, Gal-3 could have a specific diagnostic and prognostic role in patients affected by HFpEF.

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FIGURE LEGENDS

Figure 1. Correlation between Galectin-3 and C-Reactive Protein (CRP) in all patients and in patients with HFpEF.

Figure 2. Correlation between Galectin-3 and estimated Glomerular Filtration rate (eGFR) in all patients and in patients with HFpEF.

Figure 3A.Galectin 3 levels with repect to diastolic dysfunction degree in all patients a

Figure 3B. Galectin 3 levels with repect to diastolic dysfunction degree in patients with HFpEF.

Figure 4. Correlation between Galectin-3 and Left Ventricular (LV) Stiffness in all patients and in patients with HFpEF.

Figure 5. ROC Curve analysis showing the relationship among Galectin-3, B-Type Natriuretic Peptide (BNP), creatinine and outcome.