

Predictive value of plasma galectin-3 in patients with chronic heart failure

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Abstract. – **AIM:** To study the predictive value of plasma galectin-3 in patients with chronic heart failure (CHF).

MATERIALS AND METHODS: Patients with CHF (New York Heart Association functional class II-IV) caused by coronary heart disease were recruited. The levels of plasma galectin-3 and NT-proBNP were measured by enzyme-linked immuno sorbent assay. Echocardiography was performed to determine the diastolic left atrial diameter (LAD), left ventricular end-diastolic diameter (LVEDD) and left ventricular ejection fraction (LVEF). Receiver-operating characteristic (ROC) curve was used to analyze the prognostic value of galectin-3 or NT-proBNP for CHF.

RESULTS: The level of galectin-3 was significantly higher in NYHA functional class III and IV compared with that in control ($p < 0.05$ and $p < 0.01$, respectively). The level of plasma galectin-3 was positively correlated with LAD ($r = 0.271$, $p < 0.05$) and LVEDD ($r = 0.480$, $p < 0.01$), but negatively correlated with LVEF ($r = -0.683$, $p < 0.01$). The level of plasma NT-proBNP was positively correlated with LAD ($r = 0.481$, $p < 0.01$) and LVEDD ($r = 0.270$, $p < 0.05$), but negatively correlated with LVEF ($r = -0.516$, $p < 0.01$). AUC was 0.798 when the level of plasma galectin-3 was more than 7.52 ng/ml. The sensitivity to predict CHF was 62.9%, and the specificity was 90%. AUC was 0.901 when the level of plasma NT-proBNP was more than 1143 pg/ml. The sensitivity to predict CHF was 92.8% and the specificity was 85%.

CONCLUSIONS: The level of plasma galectin-3 is related to the changes of left ventricular structure and function, indicating that galectin-3 may have been involved in the process of left ventricular remodeling in CHF. The specificity of galectin-3 to predict CHF is higher than NT-proBNP, but not the sensitivity.

Key Words:

Heart failure, Galectin-3, NT-proBNP, Prognosis, Biomarkers.

Introduction

Cardiovascular diseases remain the dominant cause of death nowadays¹. Chronic heart failure (CHF), a complex clinical syndrome characterized by ventricular dysfunction, neuroendocrine activation and abnormal peripheral blood flow distribution, is a growing cause of morbidity and mortality². Moreover, diagnosis, hospitalization and related costs of patients with HF are very high, and it becomes one of the major problems of society³. Although big progress has been made in diagnosis and management of CHF, long-term prognosis is still not optimistic. Many studies show that early diagnosis and accurate judgement of severity of heart failure play an extreme important role in the treatment and prognosis.

To date, biological markers appear to have clinical importance in evaluation of the incidence and severity of heart failure in patients. However, multiple mechanisms are involved in high morbidity and mortality caused by heart failure; thus, a single marker may not be sufficient. Combination of a variety of biological markers may be more reliable⁴. Brain natriuretic peptide (BNP) and its N-terminal fragment NT-proBNP are the most commonly used biomarkers in acute or chronic heart failure these days⁵⁻⁷. Although this approach has been proven to be successful, it is known that there are kinds of mechanisms leading to heart failure in addition to myocardial overloads, and BNP and NT-proBNP values may not reflect these mechanisms. Therefore, it has become imperative to investigate more appropriate diagnostic tools to enable a more personalized management of HF.

Galectin-3 is a new biological marker that appears in recent years, which has not yet been used clinically. Galectin-3 is a macrophage- and endothelium-derived mediator actively involved

in the regulation of cardiac fibrosis and remodeling⁸⁻¹⁰. Most biological markers such as troponin are the metabolite of the heart when damage occurs, but they no longer have an impact on the heart. Galectin-3 not only serves as the “marker”, but also induces cardiac injury¹¹. Therefore, galectin-3, on the one hand, can be used in evaluation of the incidence and severity of HF, on the other hand, it can be used to predict prognosis in patients with HF (the higher the level of galectin-3, the greater the damage to heart, the worse the prognosis). Accordingly, we investigated the correlation between galectin-3 and New York Heart Association (NYHA) functional class, echocardiography and left ventricular ejection fraction (LVEF) and compared with an established biological marker NT-proBNP. Finally, we evaluated the diagnostic value of galectin-3 in patients with CHF.

Materials and Methods

Subjects

A total of 62 patients diagnosed as CHF with coronary heart disease and stable angina, were recruited in Shanghai Ninth People's Hospital between June 2010 and January 2011. A diagnosis of HF in this study conformed to the Framingham criteria and coronary heart disease and stable angina was confirmed by clinical history, physical examination, biochemical tests, electrocardiogram, chest radiography, echocardiography in conjunction with coronary angiography, myocardial radionuclide tomographic imaging and treadmill test. The exclusion criteria were having hyperthyrea, Cushing's syndrome, primary hyperaldosteronism, Addison's disease, cirrhosis, acute renal failure, paraneoplastic syndrome, subarachnoid hemorrhage, acute myocardial infarction, pulmonary heart disease, pulmonary embolism, acute muscle injury and myopathy.

Sixty-two patients were divided into three-subgroups according to the New York Heart Association (NYHA) functional class. Twenty cases of cardiac functional class II included 8 males and 12 females with the average age of 78 ± 7.9 years. Twenty-one cases of cardiac functional class III included 9 males and 12 females with the average age of 79.6 ± 8.9 years. Twenty-one cases of cardiac functional class IV included 8 males and 13 females with the average age of 77 ± 8.9 years. There were no statistical differences in the age and gender in these 3 groups.

Forty-two age-matched healthy individuals served as normal controls, which included 20 males and 22 females with the average age of 75 ± 6.5 years. There were no statistical differences in the age and gender compared with CHF groups.

Local medical Ethics Committee approved the study and all patients provided written informed consent.

Measurements of Galectin-3 and NT-proBNP by Enzyme-linked Immunosorbent Assay (ELISA)

A 3 mL of blood was collected from the median cubital vein of patients, who were on an empty stomach and in a recumbent position. The blood was anticoagulated by EDTA and centrifuged at 1,200 g for 20 min at 4°C to pellet the cells. The plasma was then removed and stored at -80°C before analysis. The level of plasma galectin-3 was determined by sandwich ELISA, using the respective dual antibody kits according to the manufacturer's instructions (BG Medicine, Waltham, MA, USA). Human NT-proBNP ELISA kit was a gift from the Department of Laboratory Medicine, Shanghai Ninth People's Hospital.

Echocardiography

Echocardiography was performed with the Cardiovascular Ultrasound System (GE VIVIDT, GE Healthcare, La Marquel, TX, USA). The frequency of ultrasonic probe was 2.5 MHz. The patients were placed in a left lateral position with quiet respiration. M-mode and 2D echocardiography was used to measure the diastolic left atrial diameter (LAD). Moreover, the left ventricular end-diastolic diameter (LVEDD) was detected in apical four-chamber section. Left ventricular ejection fraction (LVEF) was determined using biplane modified Simpson's measurements.

Statistical Analysis

Data were presented as mean \pm standard deviation (SD) and analyzed by SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Difference between two groups was compared by *t*-test and differences among three or more groups were compared by one-way analysis of variance (ANOVA). *p* < 0.05 was considered statistically significant. Association of two sets of data was evaluated with Pearson correlation analysis. Receiver-operating characteristic (ROC) curve was used to analyze the predictive value of galectin-3 or NT-proBNP for CHF.

Table I. Comparisons of age and gender in CHF and control.

	Control (n=42)	NYHA functional class			p
		II (n=20)	III (n=21)	IV (n=21)	
Age (years)	75 ± 6.5	78 ± 7.9	79.6 ± 8.9	77 ± 8.9	> 0.05
Gender (male)	20	8	9	8	> 0.05

Results

Baseline Characteristics

There were no statistical differences in the age and gender, blood glucose, total cholesterol, low-density lipoprotein (LDL), urea and creatinine between CHF and control ($p > 0.05$ Table I, Table II).

Comparison of the Level of Galectin-3 in CHF and Control

The level of galectin-3 was significantly higher in CHF compared with that in control (14.96 ± 4.87 ng/ml vs 6.37 ± 2.23 ng/ml, $F = 9.86$, $p < 0.01$, Figure 1). Furthermore, we also compared the level of galectin-3 between different NYHA functional class sub-groups and control by one-way ANOVA. The results indicated that no statistical difference of galectin-3 was present in NYHA functional class II compared with control (7.38 ± 2.35 ng/ml vs 6.37 ± 2.23 ng/ml, $p > 0.05$). The level of galectin-3 was significantly higher in NYHA functional class III compared with that in control (14.36 ± 3.94 ng/ml vs 6.37 ± 2.23 ng/ml, $p < 0.05$). The level of galectin-3 was also significantly higher in NYHA functional class IV compared with that in control (22.77 ± 5.00 ng/ml vs 6.37 ± 2.23 ng/ml, $p < 0.01$). Moreover, there were significant differences of level of galectin-3 between the sub-groups of CHF ($p < 0.05$, Figure 2).

Correlation Analysis Between the Level of Plasma Galectin-3 and LAD, LVEDD and LVEF

Pearson correlation analysis showed that the level of plasma galectin-3 was positively correlated with LAD ($r = 0.271$, $p < 0.05$, Figure 3). The level of plasma galectin-3 was positively correlated with LVEDD ($r = 0.480$, $p < 0.01$, Figure 3). The level of plasma galectin-3 was negatively correlated with LVEF ($r = -0.683$, $p < 0.01$, Figure 3).

Correlation Analysis Between the Level of Plasma NT-proBNP and LAD, LVEDD and LVEF

Pearson correlation analysis showed that the level of plasma NT-proBNP was positively correlated with LAD ($r = 0.481$, $p < 0.01$, Figure 3). The level of plasma NT-proBNP was positively correlated with LVEDD ($r = 0.270$, $p < 0.05$, Figure 3). The level of plasma NT-proBNP was negatively correlated with LVEF ($r = -0.516$, $p < 0.01$ Figure 3).

Correlation Analysis Between the Levels of Plasma galectin-3 and NT-proBNP

Pearson correlation analysis showed that the level of plasma galectin-3 was positively correlated with the level of plasma NT-proBNP ($r = 0.399$, $p < 0.01$, Figure 4).

Table II. Comparisons of clinical data in CHF and control.

Group	Fasting blood glucose (mmol/L)	Total cholesterol (mmol/L)	LDL (mmol/L)	Urea (mmol/L)	Creatinine (μ mol/L)	
Control	5.33 ± 0.46	4.44 ± 0.54	2.66 ± 0.39	7.86 ± 2.17	96.47 ± 25.28	
NYHA functional class	II	5.45 ± 1.15	4.05 ± 0.75	2.49 ± 0.62	6.79 ± 2.91	94.55 ± 40.44
	III	5.39 ± 0.98	3.88 ± 0.88	2.31 ± 0.40	8.37 ± 2.95	110.95 ± 41.15
	IV	5.23 ± 0.98	3.95 ± 0.86	2.52 ± 0.49	7.44 ± 2.28	103.29 ± 26.37
F	0.67	1.16	0.52	1.03	1.38	
P	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

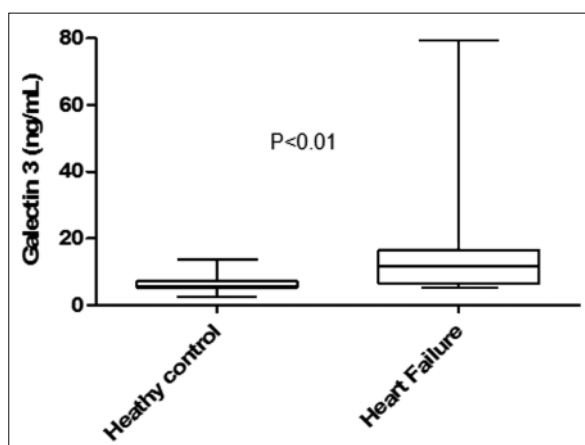


Figure 1. Comparison of the level of galectin-3 in CHF and control. The level of galectin-3 was significantly different in CHF compared with that in control ($p < 0.01$).

Analysis of the Diagnostic Value of Plasma Galectin-3 and NT-proBNP for CHF with ROC Curve

ROC curve was plotted according to the data of 62 CHF patients and 42 healthy controls. AUC was 0.798 when the level of plasma galectin-3 was more than 7.52 ng/ml. The sensitivity to predict CHF was 62.9%, and the specificity was 90% (Figure 5A). AUC was 0.901 when the level of plasma NT-proBNP was more than 1143 pg/ml. The sensitivity to predict CHF was 92.8%, and the specificity was 85% (Figure 5B).

Discussion

Cardiac remodeling is the process of structural and functional changes in the left ventricle, and is a precursor of clinical HF¹². Galectin-3 is proved to regulate macrophages function, promote fibroblasts proliferation and collagen synthesis in the process of kidney and liver fibrosis¹³⁻¹⁵. Thus, galectin-3 is thought to augment fibrosis and modulate immune response, both pivotal processes in maladaptive cardiac remodeling¹⁶. Galectin-3 expression is found to be up-regulated in patients with decompensated HF^{11,17-19}. As expected, the level of galectin-3 was significantly higher in CHF compared with that in control (14.96 ± 4.87 ng/ml vs 6.37 ± 2.23 ng/ml, $F = 9.86$, $p < 0.01$) in our study. Importantly, increased level of plasma galectin-3 was positively associated with the severity of HF, that is, plasma galectin-3 levels had statistical differences between patients with the different NYHA func-

tional class HF, especially higher in those with the NYHA functional class III and IV HF than that in healthy controls. Accumulating data also support the idea that apoptosis is involved in the transition from cardiac compensation to decompensated congestive HF²⁰, and galectin-3 inhibits apoptosis²¹, thus we assume that HF decompensation may be prompted as galectin-3 levels increases.

BNP and NT-proBNP are most widely used in diagnosis and evaluation of severity of HF currently. However, aside from myocardial stretch, other mechanisms, such as inflammation or pathways regulating cardiac contractility, may also play a role in HF, whereas these processes may not be reflected by BNP and NT-proBNP²². Besides, increased levels of NT-proBNP are associated with age, gender, obesity, heart disease, kidney dysfunction and anemia²³. The comparative studies on plasma galectin-3 and NT-proBNP in cardiac structure and function were further observed in this study. The results suggested that

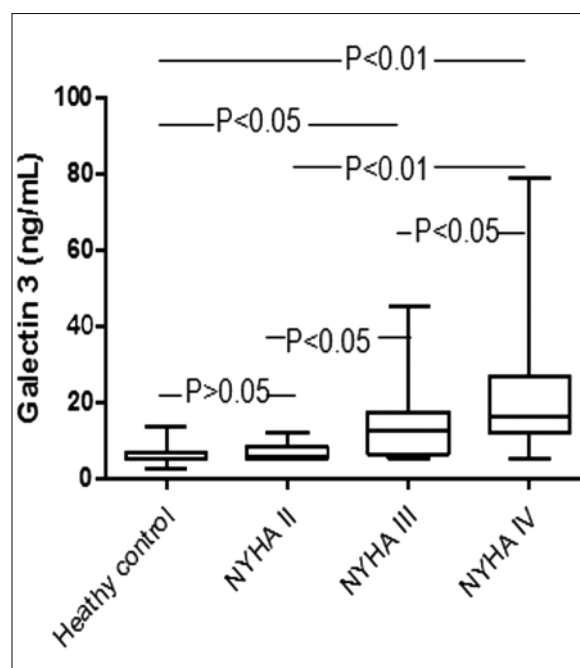


Figure 2. Comparisons of the level of galectin-3 in NYHA functional class sub-groups and control. The level of galectin-3 showed no statistical difference in NYHA functional class II compared with that in control. The level of galectin-3 was significantly higher in NYHA functional class III compared with that in control. The level of galectin-3 was significantly higher in NYHA functional class IV compared with that in control. There were significant differences of level of galectin-3 between the sub-groups of CHF.

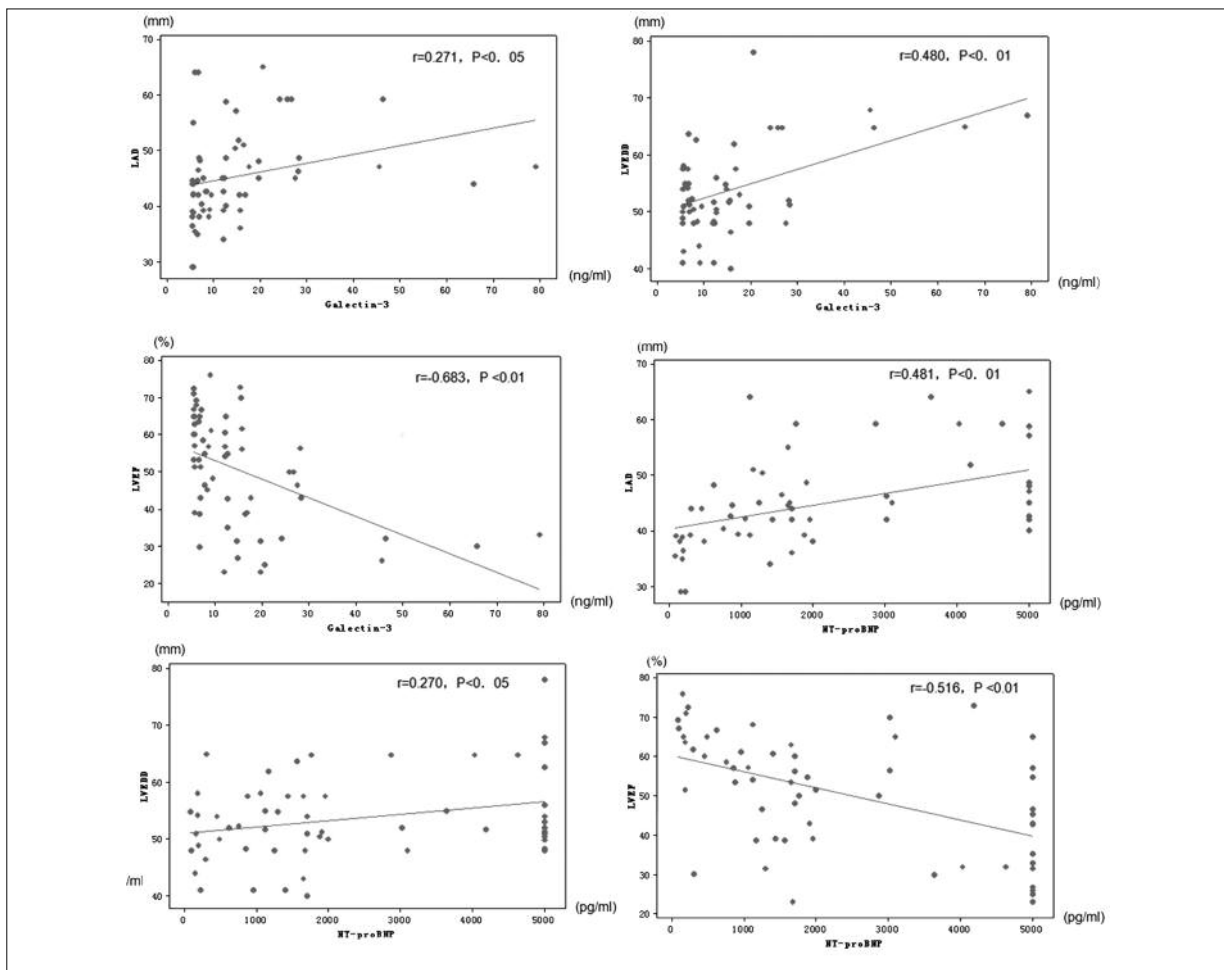


Figure 3. Correlation analysis between the level of plasma galectin-3/NT-proBNP and heart function parameters (LAD, LVEDD, and LVEF). The level of plasma galectin-3 was positively correlated with LAD ($r = 0.271$, $p < 0.05$) and LVEDD ($r = 0.480$, $p < 0.01$), but negatively correlated with LVEF ($r = -0.683$, $p < 0.01$). Similarly, the level of plasma NT-proBNP was positively correlated with LAD ($r = 0.481$, $p < 0.01$) and LVEDD ($r = 0.270$, $p < 0.05$), but negatively correlated with LVEF ($r = -0.516$, $p < 0.01$).

plasma galectin-3 and NT-proBNP concentration were positively correlated with the LAD and LVEDD, and negatively correlated to LVEF. However, the correlation between plasma galectin-3 levels and LVEDD was stronger than that between NT-proBNP and LVEDD. It indicated that galectin-3 may be involved in the process of left ventricular remodeling in CHF patients, which is in accordance with previous studies²⁴.

Furthermore, the prognostic value of galectin-3 and NT-proBNP was also compared. The findings showed that AUC was 0.798 when the level of plasma galectin-3 was more than 7.52 ng/ml. The sensitivity to predict CHF was 62.9%, and the specificity was 90%. AUC was 0.901 when the level of plasma NT-proBNP was more than 1143 pg/ml. The sensitivity to predict CHF was

92.8%, and the specificity was 85%. Generally, to be a diagnostic test, the diagnosis is of low value when ROC curve is between 0.5-0.7, and it is of medium value when ROC curve is between 0.7-0.9, when ROC curve is higher than 0.9, the diagnostic value is high²⁵. Therefore, our preliminary research suggested the diagnosis value of galectin-3 was medium, and was lower than that of NT-proBNP, but its specificity was higher than that of NT-proBNP. It has been reported that although the diagnostic value of galectin-3 is less valuable than NT-proBNP, but it still has a higher prognostic value than NT-proBNP in the 60-day mortality after heart failure hospitalization²², indicating combined biological markers were of significant value in the diagnosis of HF and risk assessment²⁶.

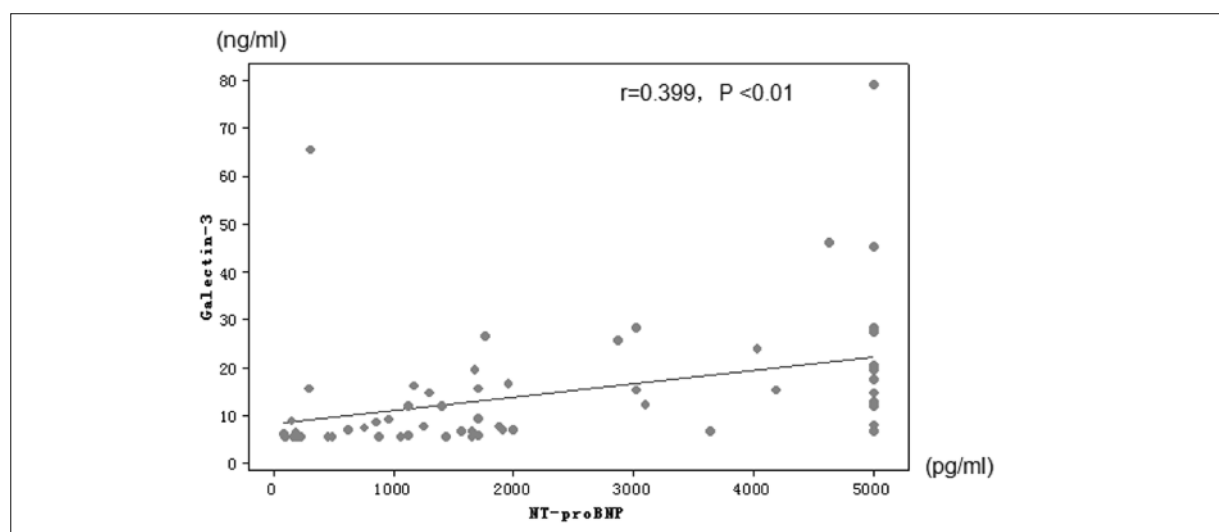


Figure 4. Correlation analysis between the levels of plasma galectin-3 and NT-proBNP. Pearson correlation analysis showed that the level of plasma galectin-3 was positively correlated with the level of plasma NT-proBNP ($r = 0.399$, $p < 0.01$).

Conclusions

The level of plasma galectin-3 is closely correlated with the heart function. Its level in CHF increases significantly as the heart function is impaired. The level of plasma galectin-3 is related to the changes of left ventricular structure and function, indicating that galectin-3 may have been involved in the process of left ventricular remodeling in CHF. The diagnosis value of galectin-3 is medium,

and is lower than that of NT-proBNP, but its specificity is higher than that of NT-proBNP.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No 30971436 and 81270376) and Science and Technology Commission of Shanghai (No 09XD1403100, 114119a8700, and 10JC1408900).

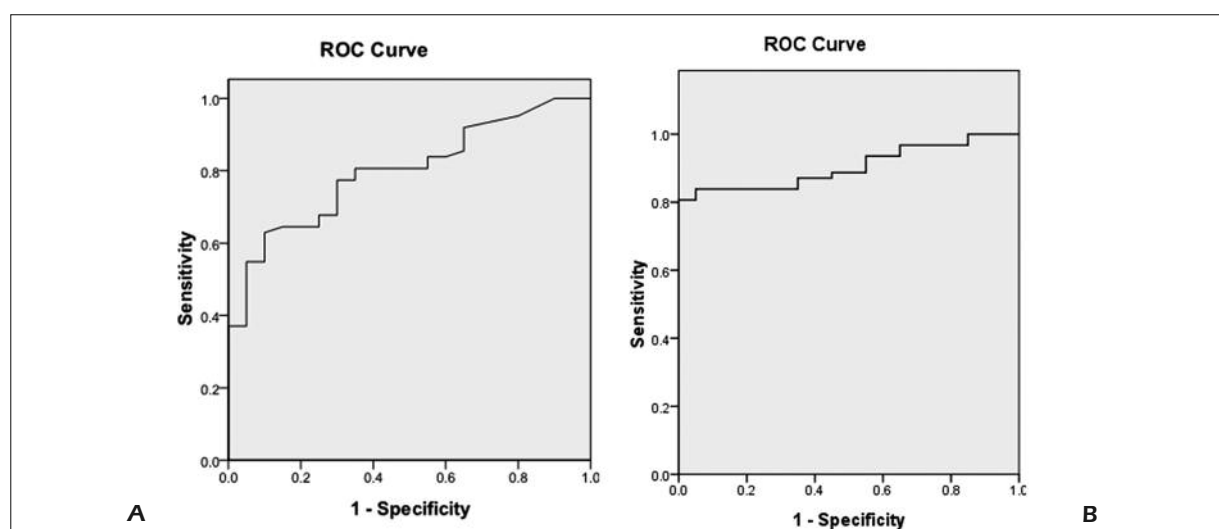


Figure 5. Analysis of the diagnostic value of plasma galectin-3 and NT-proBNP for CHF with ROC curve. ROC curve was plotted according to the data of 62 CHF patients and 42 healthy controls. **A**, Galectin-3. AUC was 0.798 when the level of plasma galectin-3 was more than 7.52 ng/ml. The sensitivity to predict CHF was 62.9% and the specificity was 90%. **B**, NT-proBNP. AUC was 0.901 when the level of plasma NT-proBNP was more than 1143 pg/ml. The sensitivity to predict CHF was 92.8% and the specificity was 85%.

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