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Original article

The Factors Influencing Galectin-3 Levels in Acute Coronary Syndrome with Decreased Left Ventricular Function

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SUMMARY

The aim of our study was to determine the factors influencing galectin-3 levels in patients with acute coronary syndrome and decreased left ventricular ejection fraction. We collected material from 37 successive patients with acute coronary syndrome and decreased left ventricular ejection fraction, of which 19 patients had atrial fibrillation, and 18 patients who were without atrial fibrillation constituted a control group. Blood samples used for the biochemical measurements were obtained on the third day from acute coronary syndrome. We used Statistical Package for Social Sciences for data analysis. A p-value less than 0.05 was considered to be a measure of statistical significance. Galectin-3 concentration is directly correlated with age and B-type natriuretic peptide level. Also, our results showed an inverse correlation between galectin-3 and total body weight, body mass index, body surface area and creatinine clearance. The following variables were found to be significant predictors of galectin-3 level: decreased left ventricular ejection fraction, total body weight, LDL concentration and body mass index. We identified factors that can predict a decrease in the left ventricular ejection fraction below 45% after acute coronary syndrome: atrial fibrillation increases the risk by almost six times, and urea concentration increases the risk by 1.2 times for each unit. Left ventricular ejection fraction below 45%, TBW, body mass index and LDL level are good predictors of galectin-3 concentration in patients with ACS and decreased left ventricular ejection fraction. Atrial fibrillation could be a predictive marker of decreased left ventricular ejection fraction.

Key words: **galectin-3, acute coronary syndrome, atrial fibrillation**

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INTRODUCTION

Galectin-3 (Gal-3) is a member of the ß-galactoside-binding lectins family, expressed in fibrotic tissues. It is associated with fibrosis and inflammation, and has been implicated in the development and progression of heart failure, and predicts increased mortality and morbidity in this condition (1). Gal-3 concentrations are not specific to the cardiovascular system and could potentially reflect other fibrotic conditions in the kidney, liver, lungs, vascular system (2). In response to circulation cytokines, part of macrophages will differentiate into alternative phenotype M2 which is important for Gal-3 production, function, and deposition of collagen in extracellular matrix, which results in adverse matrix remodeling (3).

Atherosclerosis is a major cause of acute coronary syndrome (ACS), and inflammation is known to play a key role in atherosclerosis. Permanent ligation of the left anterior descendent artery in rats is followed by increased Gal-3 expression in the infracted area and it reaches maximum in one week after myocardial infarction (MI). After acute MI, fibrosis and tissue remodeling are the leading causes of heart failure development (4).

The aim of our study was to determine the factors that influence Gal-3 levels in patients with ACS and decreased left ventricular ejection fraction (LVEF).

Patients and methods

Patient selection

This study was performed on data and material collected from 19 successive patients with ACS, AF and decreased LVEF. The LVEF, lower than 50%, was used as a criterion for the selection of patients. In 13 patients (68.4%), AF was classified as permanent/persistent, while 6 patients (31.6%) had paroxysmal AF. A control group which consisted of patients with ACS and decreased LVEF, but without AF, was formed out of 18 successive patients. There was no statistically significant difference in STEMI/NSTEMI distribution between the study and control group (42.1% vs. 44.4%, p = 1.000). They were all treated at the Clinic of Cardiology, Clinical center Niš, Serbia. The patients with previously diagnosed heart failure were excluded from the study. Standard laboratory analysis, invasive and non-invasive diagnostic procedures, and other medical records used in the study were noted on the day of admission and on the third day of hospitalization. Characteristics of the study and control groups are shown in Tables 1 and 2. In total, there were 9 male (24.3%) and 28 female subjects (75.7%). There was no statistically significant difference in gender distribution between the study and control group (p = 0.926). Their age varied from 48 to 90 years (mean age 67.11 ±10.99). Patients with AF had higher left atrium (LA) size (t = 2.145, p < 0.05), while left atrial index (LAI) parameter was similar in both groups. Besides, patients with AF had significantly higher levels of high-sensitivity C-reactive peptide (hs-CRP) (p < 0.05). Pharmacotherapy was prescribed according to the up-to-date guidelines. All the medications were prescribed in a similar manner in both study and control group (Table 2). Other parameters, such as comorbidities (diabetes mellitus type 2 (DM) and arterial hypertension (HTA)) and smoking were present with similar frequencies in these two groups.

Blood samples and biochemical measurements

Blood samples used for the biochemical measurements were obtained on the third day from ACS. Plasma was separated from the whole blood by centrifugation at 25°C temperature for 10 min at 3000g and stored at -80°C for subsequent analysis. Commercially available enzyme-linked immunosorbent assays (ELISA) were used for the determination of galectin-3, B-type natriuretic peptide (BNP) and hs-CRP plasma levels, according to manufacturer's instructions.

Statistical analysis

We used Statistical Package for Social Sciences (SPSS 21.0; Chicago, IL, USA) for data analysis. Baseline characteristics are presented as frequencies or means with standard deviations. Both parametric methods (Student's t-test), for quantitative variables and non-parametric methods (Mann-Whitney U-test), were used. Fisher`s exact test was performed to determine the association of qualitative variables. Significant predictors of the dependent variable variance were identified by standard linear, univariate and multivariate, or binary logistic regression modeling. A p-value less than 0.05 was considered to be a measure of statistical significance.

***Table 1*. Baseline characteristics of the patients**

	Atrial fibrillation	No atrial fibrillation	*t (p) or **Z (p)
Age (years)	67.21 ± 10.57	67.00 ± 11.73	0.057 (0.955)*
Total body height (cm)	175.21 ± 6.28	173.72 ± 5.75	0.751 (0.458)*
Total body weight (kg)	81.74 ± 19.69	79.39 ± 13.85	0.417 (0.679)*
Body mass index (kg/m^2)	26.55 ± 6.02	26.21 ± 3.74	0.207 (0.838)*
Body surface area (m^2)	1.98 ± 0.25	1.95 ± 0.19	0.409 (0.685)*
Left ventricular ejection fraction (%)	40.63 ± 5.91	43.78 ± 5.61	1.659 (0.106)*
Right ventricular systolic pressure (mmHg)	37.18 ± 17.27	34.83 ± 10.94	0.101 (0.920)**
End-diastolic dimension (mm)	58.33 ± 8.27	55.60 ± 6.85	0.864 (0.396)*
End-diastolic dimension index (mm/m^2)	29.37 ± 3.97	28.22 ± 3.99	0.337 (0.739)*
End-systolic dimension (mm)	41.39 ± 9.34	39.80 ± 7.71	0.352 (0.725)**
End-systolic dimension index (mm/m^2)	20.77 ± 4.46	20.18 ± 4.00	0.351 (0.725)**
Aorta (mm)	30.93 ± 4.44	33.45 ± 2.25	1.396 (0.200)*
Left atrial size (mm)	45.43 ± 3.60	41.59 ± 3.76	**2.145 (0.048)***
Left atrial index (mm/m^2)	22.36 ± 3.94	20.97 ± 14.74	0.885 (0.405)*
atrial appendage ejection fraction (%)	49.15 ± 12.09	48.67 ± 10.59	0.102 (0.920)*
End-diastolic volume (ml)	101.11 ± 28.27	91.40 ± 22.20	0.913 (0.371)*
End-diastolic volume index	50.44 ± 12.06	46.35 ± 11.39	0.848 (0.405)*
End-systolic volume (ml)	52.34 ± 22.65	47.76 ± 18.00	0.352 (0.725)**
End-systolic volume index	25.94 ± 10.16	24.16 ± 8.97	0.468 (0.639)**
Left atrial volume (ml)	60.52 ± 9.80	50.83 ± 9.48	2.087 (0.053)*
Left atrial volume index	29.95 ± 7.61	25.52 ± 3.99	1.132 (0.258)**
CHADSVASC score	4.84 ± 0.73	5.06 ± 1.35	0.575 (0.570)*
red blood cell count(10^{12}/l)	4.62 ± 0.62	4.38 ± 0.61	1.163 (0.253)*
Haemoglobin (g/l)	131.45 ± 35.81	140.44 ± 19.30	0.334 (0.738)**
Haematocrite (l/l)	42.09 ± 4.98	19.98 ± 5.43	1.236 (0.225)*
White blood cell count (10^9/l)	12.10 ± 5.34	11.38 ± 9.23	1.018 (0.309)**
Platelet count (10^9/l)	240.37 ± 71.52	253.89 ± 168.67	1.048 (0.294)**
Glucose (mmol/l)	9.68 ± 4.90	8.21 ± 2.70	0.623 (0.533)**
Creatinine (μmol/l)	119.17 ± 38.19	122.14 ± 58.71	0.380 (0.704)**
Creatinine clearance (TBW) (ml/min.)	44.28 ± 16.53	52.15 ± 17.18	1.398 (0.162)**
Creatinine clearance (TBW, TBH) (ml/min.)	60.35 ± 28.13	59.94 ± 22.25	0.049 (0.961)*
Urea (mmol/l)	8.97 ± 4.33	8.18 ± 4.48	0.927 (0.354)**
Cholesterol (mmol/l)	5.05 ± 1.86	4.90 ± 1.40	0.243 (0.808)**
Low-density lipoprotein (mmol/l)	3.12 ± 1.20	3.03 ± 1.12	0.213 (0.833)*
High-density lipoprotein (mmol/l)	0.99 ± 0.20	1.03 ± 0.28	0.851 (0.395)**
Triglycerides (mmol/l)	1.88 ± 1.99	1.82 ± 1.39	0.319 (0.750)**
Aspartate aminotransferase (U/l)	50.14 ± 57.49	63.56 ± 80.68	0.198 (0.843)**
Alanine aminotransferase (U/l)	46.42 ±44.30	31.83 ± 19.02	0.791 (0.429)**
Troponin I (ng/l)	6.08 ± 19.08	7.74 ± 17.90	0.708 (0.479)**
High-sensitivity C-reactive peptide (mg/l)	30.50 ± 26.76	11.74 ± 16.13	**2.547 (0.011)****
Creatine kinase (U/l)	836.83 ± 1391.59	726.56 ± 769.09	0.711 (0.477)**
B-type natriuretic peptide (pg/ml)	562.93 ± 554.34	387.05 ± 402.26	1.215 (0.224)**

* Student's t-test

** Mann-Whitney U-test

Table 2. **Sex, comorbidity distribution and pharmacotherapy in patients with and without AF**

	Atrial fibrillation	No atrial fibrillation	χ^2 (p)
Gender (male)	4 (21.1%)	5 (27.8%)	0.009 (0.926)
Myocardial infarction with ST-segment elevation	8 (42.1%)	8 (44.4%)	0.000 (1.000)
Diabetes mellitus type 2	4 (21.1%)	7 (38.9%)	0.683 (0.408)
Arterial hypertension	18 (94.7%)	18 (100.0%)	0.000 (1.000)
Smoking	7 (36.8%)	5 (27.8%)	0.056 (0.812)
Beta-blocker	17 (89.5%)	18 (100.0%)	0.473 (0.491)
ACE-Agiotensin converting enzyme inhibitor / Angiotensin receptor antagonist	14 (73.7%)	15 (83.3%)	0.098 (0.754)
Diuretic	12 (63.2%)	7 (38.9%)	1.316 (0.251)
Spironolacton	13 (68.4%)	16 (88.9%)	1.237 (0.266)
Statin	15 (78.9%)	18 (100.0%)	2.346 (0.126)
Heparin	18 (94.7%)	18 (100.0%)	0.000 (1.000)
Amiodarone	8 (42.1%)	2 (11.1%)	3.068 (0.080)
Isosobide-mono/dinitrate	1 (5.3%)	3 (16.7%)	0.344 (0.557)
Acetylsalicylic acid	19 (100.0%)	18 (100.0%)	
Ticagrelor/clopidogrel	19 (100.0%)	18 (100.0%)	
Trimetazidine	3 (15.8%)	3 (16.7%)	0.000 (1.000)
Molsidomine	1 (5.3%)	3 (16.7%)	0.344 (0.557)
Proton-pump inhibitor	18 (94.7%)	18 (100.0%)	0.000 (1.000)

RESULTS

Galectin-3 concentration in patients with AF

Gal-3 plasma concentration ranged from 2.01 ng/ml to 15.10 ng/ml (with average value of 8.78 ± 2.89 ng/ml). Gal-3 concentration is directly correlated with age (r^2 = 0.373, $p < 0.05$) and BNP level (r^2 = 0.331, $p < 0.05$). The inverse correlation was found with the following parameters: total body weight (TBW) (r^2 = -0.424, $p < 0.01$), body mass index (BMI) (r^2 = -0.393, $p < 0.05$), body surface area (BSA) (r^2 = -0.413, $p < 0.05$), creatinine clearance calculated from TBW and TBH (r^2 = -0.377, $p < 0.05$) and low-density lipoprotein (LDL) concentration (r^2 = -0.339, $p < 0.05$) (Table 3).

After performing a series of univariate linear regression modeling, eight independent factors stood up as significant covariates of Gal-3 plasma level (Table 4). Furthermore, these variables were selected for the multivariate regression modeling and after the exclusion of three more covariates, we obtained a statistically significant model (F = 4.890, $p < 0.01$) explaining 35.7% of Gal-3 variance (Table 5). The following covariates remained independently significant: LVEF < 45% ($p < 0.05$), TBW ($p < 0.05$), BMI ($p < 0.05$) and LDL ($p < 0.05$).

Using the method of univariate linear regression, we tried to identify the factors that can predict changes in the left atrial volume index (LAVI), the best parameter of atrial remodeling (Table 6.). Three covariates, LVEF < 45%, right ventricular systolic pressure (RVSP) and age, were significant. In a multivariate model (F = 5.228, $p < 0.05$), after excluding RVSP, which predicts 33.2% of LAVI variance, none of the dependent variables remained independently significant.

Table 3. **Correlation between galectin-3 concentration and clinical and laboratory parameters**

	r^2	p
Age (years)	**0.373**	**0.023**
Total body height (cm)	-0.204	0.225
Total body weight (kg)	**-0.424**	**0.009**
Body mass index (kg/m^2)	**-0.393**	**0.016**
Body surface area (m^2)	**-0.413**	**0.011**
Left ventricular ejection fraction (%)	-0.241	0.151
Right ventricular systolic pressure (mmHg)	0.209	0.420
End-diastolic dimension (mm)	-0.056	0.791
End-diastolic dimension index (mm/m^2)	0.347	0.090
End-systolic dimension (mm)	-0.065	0.761
End-systolic dimension index (mm/m^2)	0.207	0.331
Aorta (mm)	-0.100	0.692
Left atrial size (mm)	-0.040	0.875
Left atrial index (mm/m^2)	0.360	0.143
atrial appendage ejection fraction (%)	0.016	0.942
End-diastolic volume (ml)	-0.043	0.838
End-diastolic volume index	0.162	0.440
End-systolic volume (ml)	-0.073	0.736
End-systolic volume index	0.055	0.798
Left atrial volume (ml)	-0.020	0.937
Left atrial volume index	0.209	0.406
CHADSVASC score	0.163	0.334
red blood cell count(10^{12}/l)	0.031	0.856
Haemoglobin (g/l)	-0.175	0.299
Haematocrite (l/l)	-0.134	0.429
White blood cell count (10^9/l)	0.195	0.249
Platelet count (10^9/l)	0.189	0.263
Glucose (mmol/l)	0.124	0.466
Creatinine (μmol/l)	0.213	0.205
Creatinine clearance (TBW) (ml/min.)	-0.178	0.292
Creatinine clearance (TBW, TBH) (ml/min.)	**-0.377**	**0.021**
Urea (mmol/l)	0.311	0.061
Cholesterol (mmol/l)	-0.293	0.078
Low-density lipoprotein (mmol/l)	**-0.339**	**0.043**
High-density lipoprotein (mmol/l)	-0.225	0.181
Triglycerides (mmol/l)	-0.063	0.710
Aspartate aminotransferase (U/l)	-0.081	0.634
Alanine aminotransferase (U/l)	-0.66	0.697
Troponin I (ng/l)	-0.191	0.279
High-sensitivity C-reactive peptide (mg/l)	0.304	0.072
Creatine kinase (U/l)	0.007	0.975
B-type natriuretic peptide (pg/ml)	**0.331**	**0.046**

Table 4. **Univariate linear regression modeling of galectin-3 plasma level as a dependent variable**

	R^2	R^2 adjusted	B	SE	Beta	t	p
B-type natriuretic peptide (ng/ml)	0.109	0.084	0.002	0.001	**0.331**	2.072	**0.046**
Age (years)	0.139	0.115	0.098	0.041	**0.373**	2.379	**0.023**
Total body weight (kg)	0.180	0.157	-0.072	0.026	**-0.424**	-2.772	**0.009**
Body mass index (kg/m^2)	0.154	0.130	-0.228	0.090	**-0.393**	-2.528	**0.016**
Body surface area (m^2)	0.171	0.147	-5.371	2.002	**-0.413**	-2.683	**0.011**
Creatinine clearance (TBW, TBH) (ml/min.)	0.142	0.118	-0.043	0.018	**-0.377**	-2.408	**0.021**
Low-density lipoprotein (mmol/l)	0.115	0.089	-0.868	0.413	**-0.339**	-2.103	**0.043**
Left ventricular ejection fraction <45%	0.123	0.098	2.029	0.918	**0.350**	2.211	**0.034**

Table 5. **Multivariate linear regression modeling of galectin-3 plasma level as a dependent variable**

	Unstandardized Coefficients		Standardized Coefficients	t	p	95.0% Confidence Interval for B	
	B	SE	Beta			Lower Bound	Upper Bound
(Constant)	-33.314	26.815		-1.242	0.224	-88.077	21.450
Left ventricular ejection fraction <45%	2.162	0.807	**0.365**	2.677	**0.012**	0.513	3.811
Total body weight (kg)	-1.053	0.516	**-5.796**	-2.040	**0.050**	-2.107	0.001
Low-density lipoprotein (mmol/l)	-0.925	0.402	**-0.361**	-2.299	**0.029**	-1.746	-0.013
Body mass index (kg/m^2)	1.362	0.638	**2.233**	2.134	**0.041**	0.058	2.666
Body surface area (m^2)	46.997	26.829	3.415	1.752	0.090	-7.796	101.790

Table 6. **Univariate linear regression predicting changes in the left atrial volume index**

	R^2	R^2 adjusted	B	SE	Beta	t	p
Right ventricular systolic pressure (mmHg)	0.707	0.648	0.286	0.082	**0.841**	3.472	**0.018**
Left ventricular ejection fraction <45%	0.274	0.228	6.355	2.588	**0.523**	2.455	**0.026**
Age (years)	0.572	0.328	0.341	0.122	**0.572**	2.793	**0.013**

Table 7. **Univariate logistic regression modeling predicting decrease in the left ventricular ejection fraction below 45% as a dependent variable**

	χ^2	p	-2 log likelihood	Cox and Snell R^2	Nagelkerke R^2	B	SE	Wald	p	OR
Atrial fibrillation	6.347	0.014	43.614	0.158	0.213	1.774	0.742	5.716	**0.017**	**5.893**
Urea (mmol/l)	5.136	0.023	44.825	0.130	0.175	0.210	0.107	3.875	**0.049**	**1.234**

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.
Informed consent: Informed consent was obtained from all individual participants included in the study.

Using the method of univariate logistic regression modeling, factors that can predict the decrease in LVEF below 45% after ACS were identified (Table 7.). The presence of AF increases the risk by almost 6 times ($p < 0.05$). An increase in urea concentration increases the risk by 1.2 times for each unit ($p < 0.05$). When these variables were included into a multivariate regression model ($F = 11.196$, $p < 0.01$) that explained 26.1-35.2% of the dependent variable variance, both of them remained independently significant. The odd ratio for AF increased to 5.9 ($p < 0.05$), and for urea level it remained at 1.2 ($p < 0.05$).

DISCUSSION

Because of its involvement in cardiac fibrosis, inflammation and remodeling processes, Gal-3 is one of the emerging biomarkers in cardiac diseases.

According to our results, Gal-3 concentration is directly correlated with age and BNP level, as it is recorded in other study (5). Also, our results showed an inverse correlation between galectin-3 and total body weight, BMI, BSA and clearance creatinine. Higher levels of LDL are present in patients in AF and we found an inverse correlation with Gal-3 levels. Previous reported studies support our results (6, 7).

After performing a series of univariate and multivariate linear regression modeling, we have obtained a statistically significant model, explaining 35.7% of galectin-3 variance, using the following independently significant covariates: LVEF<45%, TBW, BMI and LDL. Increased Gal-3 levels could reflect an attempt to restore left ventricular (LV) function during the process of inflammation and fibrogenesis, or Gal-3 is up-regulated to form stiffer collagen to prevent LV dilatation. According to the study of Grandin et al., patients with Gal-3 above the median level were twice as likely to develop heart failure after ACS, and there was a weak but significant negative correlation between Gal-3 and LVEF (8) as we report in our study. The presence of Gal-3 in acute MI is possibly beneficial, but remains an adverse signal.

We tried to identify the factors that can predict changes in LAVI, the best parameter of atrial remodeling. Three covariates, LVEF < 45%, RVSP and age, were significant, but in a multivariate model, after excluding RVSP, which predicts 33.2% of LAVI variance, none of the dependent variables remained independently significant. Previous clinical findings showed that serum Gal-3 levels are correlated with LA-structural remodeling (9). Atrial size is an important determinant of AF and patients with larger atria have a higher probability for the initiation and maintenance of rotor-driven fibrillatory activity. Atrial stretch induced by increased atrial pressure may participate in AF through an effect on atrial refractoriness (10). These data are consistent with our results showing that AF is a good predictor of LA size in patients with ACS and deceased LVEF.

According to the study of Crenshaw et al., patients with AF more often had three-vessel coronary artery disease and impaired left ventricular function. The relationship between AF and MI include LV dysfunction with hemodynamic disturbances, metabolic abnormalities, excess catecholamine release or iatrogenic factors. Left atrial ischemia or infarction may be a cause, particularly in patients who develop AF within 3 hours of MI onset. Heart failure and cardiogenic shock are more frequent in the group of patients with AF (11). Using the method of univariate and multivariate logistic regression modeling, we identified factors that can predict the decrease in LVEF below 45% after ACS. The presence of AF increases the risk by almost 6 times, and increase in urea concentration increases the risk by 1.2 times for each unit. Study limitation is a small sample size. Therefore, this study was underpowered to determine a closer relationship between galectin-3 concentration and AF preexistence.

CONCLUSION

Left ventricular ejection fraction below 45%, TBW, BMI and LDL level are good predictors of galectin-3 concentration in patients with ACS and decreased LVEF. Atrial fibrillation could be a predictive marker for decreased left ventricular ejection fraction.

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Faktori koji utiču na nivoe galektina-3 kod akutnog koronarnog sindroma sa smanjenom funkcijom leve komore

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SAŽETAK

Cilj naše studije bio je da odredimo faktore koji utiču na nivo galektina-3 kod bolesnika sa akutnim koronarnim sindromom i sniženom ejekcionom frakcijom leve komore. Sakupljen je materijal od 37 bolesnika sa akutnim koronarnim sindromom i sniženom ejekcionom frakcijom leve komore, od kojih je 19 imalo atrijalnu fibrilaciju, a 18 bez atrijalne fibrilacije je predstavljalo kontrolnu grupu. Uzorci krvi su uzeti trećeg dana od pojave akutnog koronarnog sindroma. Za analizu podataka smo koristili SPSS (Statistical Package for Social Sciences) softver. P-vrednost manja od 0,05 je smatrana statistički značajnom. Vrednosti galektina-3 su direktno korelirale sa godinama starosti i vrednostima natriuretskog peptide tipa B. Sledeće promenljive su bile značajni prediktori nivoa glaktina-3: snižena ejekciona frakcija leve komore, telesna težina, nivo LDL i indeks telesne mase. Takođe, naši rezultati su pokazali negativnu korelaciju galektina-3 sa ukupnom masom tela, indeksom telesne mase, ukupnom telesnom površinom i klirensom kreatinina. Identifikovali smo faktore koju mogu predvideti pad ejekcione frakcije leve komore ispod 45% nakon akutnog koronarnog sindroma: atrijalna fibrilacija povećava rizik 6 puta, a povećanje koncentracije uree za svaku jednicu 1,2 puta. Ejekciona frakcija leve komore manja od 45%, telena težina, indeks telesne mase i nivo LDL su dobri prediktori koncentracije galektina-3 kod bolesnika sa AKS i sniženom ejekcionom frakcijom. Atrijalna fibrilacija moze poslužiti kao prediktivni marker sniženja ejekcione frakcije leve komore.

Ključne reči: **galektin-3, akutni koronarni sindrom, atrijalna fibrilacija**