Galectin-3 in Patients with Atrial Fibrillation and Restored Sinus Rhythm

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Abstract

Introduction: Cardiac fibrosis is the hallmark of atrial remodeling in atrial fibrillation. Galectin-3 (Gal-3) is a biomarker of fibrosis. It is well studied in heart failure, but the data about its role in atrial fibration are sparse.

Aim: The aim of the study was to evaluate the levels of Gal-3 in patients with atrial fibrillation after sinus rhythm restoration, to examine the association between this biomarker and other factors for developing atrial fibrillation and to assess its prognostic role.

Materials and methods: We included 67 patients (35 male) at the mean age of 67.36±7.25 years, with Gal-3 test after sinus rhythm restoration, a subgroup of participants in placebo-controlled randomized clinical trial of treatment with spironolactone. They were followed up for atrial fibrillation recurrence and hospitalizations. The effect of demographic parameters and other factors on Gal-3 levels were evaluated before and one year after treatment.

Results: Mean Gal-3 at baseline was 16.9±6.8 ng/ml. Higher levels of Gal-3 were associated with female gender (p=0.008), increasing age (p=0.005), renal dysfunction (p<0.0001) and gout (p=0.002). Higher thromboembolic risk as assessed by CHA2DS2-VASc score was significantly related to Gal-3. The levels of biomarker did not affect the number of atrial fibrillation recurrences (p=0.9) and hospitalizations. No correlation was found with treatment with spironolactone, antiarrhythmic and antihypertensive drugs.

Conclusions: Higher Gal-3 in atrial fibrillation was associated with female sex, renal dysfunction, and history of gout. The levels of Gal-3 were not related to rhythm control. Treatment with spironolactone did not affect the biomarker of fibrosis Gal-3 in AF patients. Higher Gal-3 was related to high embolic risk.

Keywords
atrial fibrillation, fibrosis, galectin-3

INTRODUCTION

Despite the progress that has been made in managing atrial fibrillation (AF), the pathophysiology of this condition is not fully understood. Indeed, AF is a heterogeneous disorder and the structural, electrical, and contractile remodeling are important synergic factors for the formation of the arrhythmia substrate. Cardiac fibrosis is the hallmark of arrhythmogenic structural remodeling. Such fibrosis is closely associated with formation and redistribution of the extracellular matrix. This may lead to myocyte slippage, tissue heterogeneity, ventricular dyssynchrony or
dilatation and contractile dysfunction.\textsuperscript{3,5-7} The resulting change is seen in the myocardial geometry and development of atrial cardiomyopathy.\textsuperscript{3,9}

Galectin-3 (Gal-3) is a soluble β-galactoside binding lectine that plays an important role in the modulation of fibrosis, inflammation and immune responses.\textsuperscript{10,11} It is a well-known biomarker for risk stratification and prognostication in heart failure (HF) patients, but the data about the role of Gal-3 in AF are sparse. Some studies have shown that high circulating Gal-3 levels correlate with the degree of atrial fibrosis\textsuperscript{12} and predict incident AF\textsuperscript{13,14}.

**AIM**

The aim of the study was to evaluate the levels of Gal-3 in patients with AF after sinus rhythm restoration, to examine the association between this biomarker and other factors for developing AF and to assess its prognostic role.

**MATERIALS AND METHODS**

**Study design**

This is a randomized, single-center, clinical trial of the effect of mineralocorticoid receptor antagonist (MRA) spironolactone on top of standard treatment in patients with AF after sinus rhythm restoration on the recurrence of the arrhythmia, hospitalizations and on the changes in Gal-3 after 12 months, compared to the control group who were treated according to the ‘usual care’ following rhythm control. The patients were followed up for 1 year and had follow-up visits at 14 days, 1 month, 3 months, 6 months, 9 months, and at 12 months.

**Patient selection**

The diagnosis of AF was confirmed by ECG criteria when the patient had been hospitalized or had visited the Emergency Department of the hospital and had the sinus rhythm restored during that time, spontaneously or after medical or electrical cardioversion. The type of AF was classified according to the ESC Guidelines on AF 2010 and 2016.\textsuperscript{15-17} The duration of current AF episode was between 1 and 45 days. The median duration of the AF to enrolment was 196 days, range from 0 days to 23.9 years. In all patients, the thromboembolic risk was calculated according to CHA\textsubscript{2}-DS\textsubscript{2}-VASc (congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, stroke or transient ischemic attack, vascular disease, age 65 to 74 years, sex category) score.\textsuperscript{18} The participants were also divided without clinical stroke risk factors (0 in males, 1 in females) and with risk factors (≥1 in a male or ≥2 in a female).

Inclusion criteria were as follows: age ≥55 years, restored sinus rhythm after an episode of paroxysmal/persistent AF, signed informed consent. Exclusion criteria included history of clinical and echocardiographic evidence of chronic HF NYHA class III-IV; open heart surgery during the last 3 months for any indication; survivors of acute myocardial infarction and left ventricular dysfunction within 3 months of randomization; pregnancy; drug and alcohol abuse; presence of severe progressive concomitant disease with life expectancy less than 1 year; chronic kidney disease defined as serum creatinine more than 200 mmol/l or eGFR less than 40 ml/min/1.73 m\textsuperscript{2}; Child C liver cirrhosis; treatment with powerful CYP3A4 inhibitors or inductors; serum potassium levels >5 mmol/l at screening; hypersensitivity towards MRA; metabolic acidosis; known thyroid pathology with lab results consistent with hyper- or hypothyroidism.

**Outcome measures**

At each visit, the patients were interviewed for episodes of recurrent arrhythmia. Information about their vital status or other hospitalizations was also collected. The etiology was considered to be due to CVD or other causes by the investigators (AK, YY).

**Laboratory tests**

Blood for Gal-3 determination was collected at baseline (on the next day after sinus rhythm restoration) and one year after. Serum Gal-3 levels were determined using enzyme-linked immunosorbent assay kit for quantitative measurement (Galectin-3 AssayTM, REF# 12642-04, 12684 BG Medicine, Waltham, MA, USA) and were measured using StatFax 3200 microplate reader (Awareness Technology, Inc., USA).

In all patients, blood for biochemistry was taken usually after at least 12 hours fasting at visits 1, 2, 3, 5, and 7 or whenever necessary for evaluating serum creatinine, potassium and sodium. The estimated glomerular filtration rate (eGFR) as an indicator of renal function, was calculated using a formula CKD-EPI formula.\textsuperscript{19}

**ECG**

Standard 12-lead ECG was done at each visit.

**Measurement of coronary artery calcium score (CACS)**

CACS was measured in 41 patients using ECG-triggered non-contrast computer cardiac tomography and the Agatston score method.\textsuperscript{20} Coronary calcium lesions were defined as having a threshold ≥ 130 HU and an area of the plaque. The computer tomograph used was Siemens Somatom Definition (Dual Source). The risk of obstructive coronary artery disease (CAD) was evaluated using pre-test probability calculator (CAD consortium).
**Statistical analyses**

All continuous variables were presented as means ± standard deviation for relatively normally distributed and as median (interquartile range) for those with deviation from normality. When approximately normal distribution was present, the independent variables were compared by Student's t-test or ANOVA test for repeated measurements, and paired t-test or one-sample t-test were applied for the differences in variables between the end and first visits. Because of the right-skewed distribution of Gal-3 values, we made a log transformation to improve the non-normal distribution. For categorical variables, absolute values and percentages were presented and the chi-square test or Kendall's τ-analysis were used to test the null hypothesis. When the expected cell numbers were smaller than 5, then the exact Fisher's test is applied. P-value <0.05 was used for significance testing. Correlation analyses using the Pearson's or Spearman's method were performed to test the relation between different continuous or categorical variables.

Linear regression analyses were separately performed, with log Gal-3 at baseline, at the end of the study and for the difference between the visits as the dependent variable. In case of significance of the univariable testing, multivariate linear regression models were applied, with age, gender BMI and baseline Gal-3 values as covariates to adjust for. All analyses were performed on SPSS® version 19 (SPSS, Texas, USA).

**Ethical approval**

The study was approved by local Committee of Medical Ethics of the St Marina University Hospital, Varna and complied with the Declaration of Helsinki. Informed consent was obtained by all patients.

**RESULTS**

**Study population**

The majority of the patients had their sinus rhythm restored by medical cardioversion (79%), 18% received electrical cardioversion. Overall, 67 patients with AF and restored sinus rhythm were included in the sub study. Gal-3 was measured at baseline in n=67 and in n=62 both at baseline and after one year of follow-up.

The males were non-significantly more than the females – 35 (52%) vs. 32 (48%), \( p=0.91 \). The mean age was 67.36±7.25 years (55-83 years). Baseline group characteristics were presented and the chi-square test or Kendall's τ-analysis were used to test the null hypothesis. When the expected cell numbers were smaller than 5, then the exact Fisher's test is applied. P-value <0.05 was used for significance testing. Correlation analyses using the Pearson's or Spearman's method were performed to test the relation between different continuous or categorical variables.

**Table 1. Baseline demographic, clinical, laboratory and echocardiographic parameters of study population**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>29.90</td>
<td>5.95</td>
</tr>
<tr>
<td>sBP, mm Hg</td>
<td>127.57</td>
<td>14.01</td>
</tr>
<tr>
<td>dBP, mm Hg</td>
<td>76.07</td>
<td>7.54</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>63.39</td>
<td>10.52</td>
</tr>
<tr>
<td>Creatinine, mmol/L</td>
<td>85.71</td>
<td>17.23</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>72.59</td>
<td>14.46</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>138.91</td>
<td>17.41</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.10</td>
<td>0.44</td>
</tr>
<tr>
<td>LA area, cm²</td>
<td>21.49</td>
<td>3.48</td>
</tr>
<tr>
<td>LA volume index, mL/BSA</td>
<td>34.90</td>
<td>9.60</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>60.22</td>
<td>6.06</td>
</tr>
<tr>
<td>E/A ratio, mitral valve</td>
<td>1.33</td>
<td>1.12</td>
</tr>
</tbody>
</table>

BMI: body mass index; sBP: systolic blood pressure; dBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; LA: left atrium; LV: left ventricle; BSA: body surface area; E/A: early (E) to late (A) ventricular filling velocities

**Galectin-3 in renal dysfunction and in gout**

The mean eGFR was 70.4±15.3 ml/min/1.78 m², the mean serum creatinine – 86.5±17.4 mc mol/l. Gal-3 levels were elevated in patients with renal dysfunction. There was a weak positive correlation with baseline creatinine and significant negative correlation with baseline eGFR (\( r=-0.47 \), \( p<0.001 \)) (Fig. 2). Patients with history of gout had significantly higher values of Gal-3 compared with patients without gout (28.5±15 vs. 16±5.5, \( p=0.002 \)).

There was no important association between Gal-3 and treatment with spironolactone (\( p=0.69 \)), diabetes mellitus (\( p=0.33 \)), dyslipidemia (\( p=0.73 \)), and smoking (\( p=0.4 \)).

**Gal-3 and rhythm control**

In patients with paroxysmal AF, Gal-3 was non-significantly higher than in persistent AF (\( p=0.25 \)). The levels of the biomarker did not affect the number of AF recurrences (\( p=0.9 \)) and the hospitalizations. There was no significant difference in the change in Gal-3 in one year between patients with or without AF recurrences – 1.0 ng/ml (\( p=0.26 \)).

The sub-analysis on Gal-3 in relation to type of restoration of sinus rhythm revealed that numerically Gal-3 was the highest in the spontaneously restored rhythm group – 28.25, followed by medically treated – 17.11 ng/ml and was lowest in the electrically cardioverted but no significant distinction between the groups was found (\( p=0.137 \), ANOVA).
Gal-3 and thromboembolic risk as assessed by CHA2DS2-VASc score

The embolic risk was related significantly to the baseline Gal-3 levels. Patients with risk factors for stroke had significantly higher levels of the biomarker at baseline (17.3±6.8 ng/ml vs. 10.4±1.9 ng/ml, p<0.001). This relationship remained until the end of the study (17.7±5.6 vs. 10.5±2.0 ng/ml, p=0.001). Univariate regression analysis showed a strong linear relationship between the log-transformed values of Gal-3 and CHA2DS2-VASc score: β-coefficient 0.102, 95% CI, 0.054–0.150, p<0.0001 (Fig. 3).

In a multivariate model, with the baseline log Gal-3 as the dependent variable, the significant variables to predict the increase of the biomarker for fibrosis were the CHA2DS2-VASc score of the patients with AF and eGFR (Table 2). Backward selection of the variables in the model was used.
Galectin-3 in Atrial Fibrillation

Figure 3. Relationship between the logarithmically transformed values of Gal-3 and CHA2DS2-VASc score, N=67.

Table 2. Linear multivariable regression model with logarithmically transformed values of Gal-3 as dependent variable and log Gal-3 at baseline, CHA2DS2-VASc score, BMI and eGFR as independent variables.

<table>
<thead>
<tr>
<th>Model</th>
<th>β coefficient</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>3.1±0.32</td>
<td>2.469-3.747</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHA2DS2-VASc score</td>
<td>0.073±0.024</td>
<td>0.024-0.122</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.002±0.006</td>
<td>-0.011-0.014</td>
<td>0.789</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.008±0.003</td>
<td>-0.013-0.003</td>
<td>0.003</td>
</tr>
</tbody>
</table>

CHA2DS2-VASc: congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, stroke or transient ischemic attack, vascular disease, age 65 to 74 years, sex category; BMI: body mass index; eGFR: estimated glomerular filtration rate.

Galectin-3 and risk for coronary artery disease

The baseline values and the change of Gal-3 after 1 year were comparable to patients with (CACS>0) and without (CACS=0) subclinical atherosclerosis. There was an increase of the biomarker at 12 months in patients with moderately high risk for obstructive CAD with 2.57 ng/ml vs. practically no change (-0.03 ng/ml) in low risk patients, although this was not significant (p=0.12).

DISCUSSION

Values of Gal-3 in our AF patients were significantly higher in comparison with the values in healthy individuals of the same age from Framingham Offspring cohort (n=1072)\textsuperscript{13}, measured with the same test (BG medicine), although these are different cohorts. This was valid especially for Gal-3 concentration in women over 60 years: at the 50th percentile – age group 60 years – 17.2 ng/ml vs. 13.5 ng/ml, 70 years – 16 ng/ml vs. 14.8 ng/ml, 80 years 21.8 ng/ml vs. 16.2 ng/ml and in men: aged 50–59 – 13.1 ng/ml vs. 11.8, and over 80 years old 18.3 ng/ml vs. 15.5 ng/ml (p<0.05, Mann-Whitney U test). We found that Gal-3 increases with age and is higher in females than in males.

The association between Gal-3 levels and renal function in our trial confirms the data from other studies.\textsuperscript{21-23} The levels of Gal-3 in our patients with history of gout were significantly higher compared with the levels in patients without gout. Interestingly, we found strong relation between Gal-3 and CHA2DS2-VASc score both at baseline and end of study.

The data about the role of Gal-3 in patients with AF are heterogeneous. Chen et al.\textsuperscript{21} evaluated 131 patients, hospitalized for AF and found that Gal-3 has a significant correlation with new onset AF (β=0.2, p<0.05). Somnez et al.\textsuperscript{24} also found significantly elevated Gal-3 in AF patients: 1204 pg/ml (1166–1362) vs. 1166 pg/ml (1126–1204), (p=0.001). Ho et al.\textsuperscript{13} demonstrated an association between higher circulating Gal-3 concentrations and increased risk of developing AF; however, this study failed to demonstrate that
Gal-3 levels could be used to predict AF after adjustment for traditional clinical risk factors. In contrast, other studies have not found evidence for association between elevated Gal-3 and AF. Zakeri et al. in the RELAX trial found only slightly increased Gal-3 concentrations in patients with HF with preserved ejection fraction (HFP EF) and AF vs. patients with HFP EF in sinus rhythm 14.3 (11.7–19.7) vs. 13.6 (10.8–16.9), \( p=0.15 \).

Our results regarding the Gal-3 association with age and sex category are in agreement with the results of De Boer et al. They evaluated Gal-3 levels in the general population, using blood samples from the participants in PREVEND (Prevention of Renal and Vascular Endstage Disease) study. The researchers found a strong positive correlation between the Gal-3 levels and the product age*sex in the analysis – \( P = 2.39e-04 \). Ho et al. also demonstrated significant elevation of the biomarker with the age ( \( p<0.0001 \)), and in female subjects ( \( p<0.05 \)) with mean Gal-3 concentrations 14.3 ng/ml vs. 13.1 ng/ml in males.13,22

Different mechanisms for Gal-3 elevation in renal impairment are discussed. It is possible that Gal-3 has renal excretion and is a marker for renal dysfunction. There is also probably increased renal production of Gal-3 and the profibrotic effect of the biomarker leads to worsening of renal function. There are some interesting data that Gal-3 may protect the kidney from ischemia/reperfusion injury, also in case of chronic kidney disease. It is suspected that in patients with cardiac diseases, Gal-3 is produced from the heart, kidneys and other organs in the state of general inflammation.31,32

Up to now, there have been no data in the available literature about the levels of Gal-3 in patients with gout. The association between the biomarker and the disease is not surprising. It is well known that patients with gout have an elevated cardio-vascular risk.33,34 On the other hand, Gal-3 is a marker of fibrosis, but also of inflammation.

There are few reports on the correlation between Gal-3 and CHA2DS2-VASc score. Clementy et al. found a positive correlation between the biomarker and thromboembolic score. The elevated levels of Gal-3 are marker of activation of fibrosis, which is also a pro-inflammatory state. This status predisposes for thrombus formation and increases the risk for stroke. Thus, the biomarker could be used for estimation of the patients risk profile.

**Limitations of the study**

Galectin-3 concentrations are not specific to the cardiovascular system and other fibrotic processes could cause their elevation. Examples of this are cancers and inflammatory diseases and they are described in the literature. The number of patients in this sub-study is relatively small and is not great enough to show differences in Gal-3 in accordance with the treatment with spironolactone or other important risk factors. The relationship between gout and Gal-3 is based only on the history of the disease and without measuring the uric acid concentrations. The latter correlation may give a more in-depth rationale for this hypothesis.

**CONCLUSIONS**

We found that female sex, renal dysfunction, and history of gout were associated with higher circulating Gal-3 levels in AF. The levels of Gal-3 were not related to rhythm control. Treatment with spironolactone did not affect the biomarker of fibrosis Gal-3 in AF patients. Higher Gal-3 was related to high embolic risk. Further studies are needed to clarify the role of Gal-3 in AF.

**Funding**

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**REFERENCES**


Галектин-3 у пациентов с фибрилляцией предсердий и восстановлением синусового ритма

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Резюме

Введение: Сердечный фиброз является признаком ремоделирования предсердий при фибрилляции предсердий. Галектин-3 (Гал-3) является биомаркером фиброза. Он хорошо изучен при сердечной недостаточности, но данные о его роли при фибрилляции предсердий немногочисленны.

Цель: Целью исследования было оценить уровни Гал-3 у пациентов с фибрилляцией предсердий после восстановления синусового ритма, изучить взаимосвязь между этим биомаркером и другими факторами развития фибрилляции предсердий и оценить его прогностическую роль.

Материалы и методы: В исследование были включены 67 пациентов (35 мужчин), средний возраст которых составил 67.36 ± 7.25 года. с тестом Гал-3 после восстановления синусового ритма, подгруппа участников плацебо-контролируемого рандомизированного клинического исследования лечения спиронолактоном. За ними наблюдали на предмет рецидива фибрилляции предсердий и госпитализаций. Влияние демографических параметров и других факторов на уровни Гал-3 оценивали до и через год после лечения.

Результаты: Средний исходный уровень Гал-3 составлял 16.9 ± 6.8 ng/ml. Более высокие уровни Гал-3 были связаны с женским полом (p=0.008), нежелателым возрастом (p=0.005), почечной дисфункцией (p<0.0001) и подагрой (p=0.002). Более высокий риск тромбоэмболии, который оценивался по шкале CHA2DS2-VASc, был значительно связан с Гал-3. Уровни биомаркеров не повлияли на количество рецидивов фибрилляции предсердий (p=0.9) и госпитализаций. Не было обнаружено корреляции с лечением спиронолактоном, антиаритмическими и гипотензивными препаратами.

Заключение: Более высокие уровни Гал-3 при фибрилляции предсердий были связаны с женским полом, почечной дисфункцией и подагрой по анамнезу. Уровни Гал-3 не были связаны с контролем ритма. Лечение спиронолактоном не влияло на биомаркер фиброза Гал-3 у пациентов с ФП. Более высокие уровни Гал-3 были связаны с более высоким риском эмболии.

Ключевые слова

фибрилляция предсердий, фиброз, галектин-3