Plasma galectin-3 as a biomarker for clinical staging of heart failure: a cross-sectional evaluation of 100 cases

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Abstract

Objectives. To evaluate the plasma galectin-3 concentration associated with the severity of HF and its use as a biomarker for clinical staging of heart failure (HF).

Method. This was a cross-sectional study, in which 100 HF cases diagnosed by clinical parameters and echocardiography were included and subgrouped into NYHA classes (I–IV) based on clinical severity and functional limitations. Plasma galectin-3 was measured with respect to these subgroups.

Results. The median plasma galectin-3 concentration in pg/mL was 82.7 (95% confidence interval: 64.5–112.7), 267.2 (214.3–293.5), 694.8 (626.4–902.4), and 1530.4 (1443.1–2384.4) in NYHA class I, II, III, and IV subgroups, respectively (p < 0.05). The proposed galectin-3 concentrations in mild, moderate, and severe HF were 100–460, 460–1170, and >1170 pg/mL, respectively. Galectin-3 was negatively correlated with LV Ejection fraction (EF) by Simpson’s biplane method (r=-0.634, p<0.001). Pro BNP showed that the level of plasma galectin-3 was positively correlated with the level of plasma NT pro BNP (r = 0.878, p < 0.001).

Conclusions. The plasma galectin-3 concentration showed progressive increase with increasing severity of HF; therefore, it may be used in clinical staging of the disease. Clin Ter 2019; 170(4):e267-271. doi: 10.7417/CT.2019.2146

Key words: Apoptosis, biomarker, cardiac fibrosis, galectin-3, heart failure, staging of heart failure

Introduction

Heart failure (HF) is a common and highly morbid cardiovascular disorder associated with perturbations in cardiac structure and function. Incidence of HF is gradually increasing. For individuals >40 years, the lifetime risk of developing HF has been estimated to be approximately 20%. The incidence of HF is highest in population >65 years,1 which is rapidly growing, ensuring an epidemic of HF that will continue to grow as the population ages.2 According to Boon et al.,3 the prevalence of HF rises from approximately 1% among patients aged 50–59 years to approximately 5%–10% among those aged 80–89 years.

Good management of HF depends on accurate diagnosis.3 Traditionally the diagnosis of HF is clinically based on signs, symptoms, chest radiographs, and response to therapy. Because most of these findings are non-specific, the diagnosis of HF is often extremely difficult, and both under- and over-diagnosis is common, particularly among the elderly population, obese patients, and patients with underlying lung disease.2,3 In addition to diagnosis, the clinical staging of HF with respect to its severity is very important for proper management.3 To date, diagnosis is primarily based on clinical parameters. The New York Heart Association (NYHA) has developed a system for the clinical staging of HF that ranges from the least (class I) to most severe forms (class IV) on the basis of some clinical parameters.4,5 Today, studies have focused on an inexpensive, specific, sensitive, readily available, and easily interpretable aid for the diagnosis and staging of HF, irrespective of the underlying etiopathophysiology.3

One of them is pro brain natriuretic peptide (NT pro BNP) and it has effect on the pathophysiological mechanisms of HF appear to be the results of interaction between cardiac remodeling, neurohormonal peptides.6 Galectin-3, a member of the galactic family, is a 30 kDa protein. Galectin-3 is an emerging marker of cardiac fibrosis, which is an outcome of HF.7 Now, increasing evidence is in consensus with using plasma galectin-3 as a diagnostic and prognostic biomarker for HF.7,8 However, its use as a marker for clinical staging of HF is yet to be established conclusively.

Abbreviations list

HF: Heart failure
NYHA: The New York Heart Association
NTpro-BNP: N-terminal pro-brain natriuretic peptide
CI: Confidence interval
EF: Ejection fraction

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Therefore, in the present study, we aimed to evaluate the role of plasma galectin-3 as a biomarker for the clinical staging of HF.

Materials and methods

Study design and patients

This was a cross-sectional, single-arm study performed in the Cardiology Clinics between July 2013 and June 2014. During the study period, 100 consecutive patients with HF (mean age, 41.1 years; age range, 10–70 years; male: female, 61:39). The exclusion criteria were ischemic heart disease, renal failure, non-cardiac fluid overload, thyroid disorders, or liver diseases.

The study was conducted according to the latest version of Helsinki Declaration. All subjects were informed and signed written consent prior to their enrollment.

Study procedures

Diagnosis of HF was performed on the basis of clinical and echocardiographic findings, and patients were classified into NYHA subgroups (class I–IV) according to clinical severity and functional limitation.5 9 A study-specific questionnaire was used to collect data on demographics and clinical findings. Trans-thoracic echocardiography (TTE) was performed for all patients in both supine and left lateral position using VIVID 7 GE system with tissue Doppler imaging (TDI) capability. All cases were examined using multifrequency (2.5–3.5 MHZ) Matrix probe. The LV ejection fraction was calculated by a modified biplane Simpson’s method from apical 4- and 2 chambers views. Blood samples were aseptically collected from each patient, and plasma was eventually separated for the measurement of plasma galectin-3 using the galectin-3 assay, which is an enzyme-linked immune-sorbent assay developed by BG Medicine (BG Medicine, Inc., Waltham, USA). This assay quantitatively measures the concentration of human galectin-3 levels in EDTA plasma. It has high sensitivity (lower limit of detection, 1.13 ng/mL) and exhibits no cross-reactivity with collagens or other members of the galectin family. Also same blood samples used to evaluate for NT-pro BNP. The human NT-pro BNP ELISA kit (Wuhan ElAab Scince Co., Ltd.) is based on the competitive binding enzyme immunoassay technique. Monoclonal antibody specific for NT-pro BNP has been pre-coated onto amicrotiter plate. Standards and samples are pipetted into the wells and any NT-pro BNP present is bound by the immobilized antibody. The level of NT-pro BNP was determined according to the manufacturer’s instructions. Commonly used HF medication like angiotension-converting enzyme inhibitors, beta-blockers, spironolactone, furosemide, acetylsalicylic acid, warfarin, and digoxin do not show interference with the assay.11

Serum galectin-3, NT-pro BNP levels and LV ejection fractions of NYHA subgroups (class I–IV) were compared for evaluating the association between the severity of HF and serum galectin-3 level. Furthermore, plasma galectin-3 levels to be used clinically for the assessment of the severity of HF were proposed.

Statistical analysis

Study data were summarized using descriptive statistics (e.g., mean, median, standard deviation, range, 95% confidence interval [CI], frequency, and percentage). Plasma galectin-3 concentrations of four NYHA subgroups were compared using one-way analysis of variance, and for significant differences Bonferroni post-hoc multiple comparison test was performed. The proposed plasma galectin-3 levels for each NYHA class of HF were defined by bridging gaps between 95% CIs of consecutive classes, with half of the gaps on either side, and compromising class limits to nearest zero or five with a cut off level of 100 pg/mL. Data were analyzed using the SPSS software package for Windows (Statistical Package for Social Sciences, version 12.0, SPSS Inc., Chicago, Illinois, USA). Statistical level of significance was defined to be p < 0.05. NT-pro BNP levels and LV ejection fractions were presented as mean ± standard deviation (SD) and analyzed by SPSS 12. Difference between two groups was compared by t-test and differences among 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 and Spearman’s test for correlation analysis.

Results

The study population was comprised of patients with NYHA class I–IV HF. The number of patients with class I HF was lowest, whereas a similar number of patients were present in each one of class II–IV (Table 1). The median plasma galectin-3 concentration was 82.7 pg/mL (95% CI: 64.5–112.7), 267.2 pg/mL (214.3–293.5), 694.8 pg/mL (626.4–902.4), and 1530.4 pg/mL (1443.1–2384.4) in NYHA class I, II, III, and IV subgroups, respectively. There was a significant progressive rising trend in plasma galectin-3 concentration as NYHA class, i.e., the severity of HF increases (p < 0.05, Table 1). Multiple comparisons between NYHA classes showed that, although there was no significant difference in galectin-3 level between class I and II HF, as the class increases galectin-level became significantly higher than the class I and previous class (Table 2).

On analysis of 95% CI of plasma galectin-3 concentrations in different NYHA classes, galectin-3 concentrations in mild, moderate, and severe HF were proposed to be 100–460, 460–1170, and >1170 pg/mL, respectively (Table 3).

There were statistically significant differences among ejection fractions in heart failure subgroups (p < 0.001, Table 4) and the level of plasma galectin-3 was negatively correlated with LVEF (r = −0.634, p < 0.001, Table 5). Also we found that NT-pro BNP plasma levels statistically different in heart failure subgroups (p < 0.001, Table 4). Correlation analysis between the levels of plasma galectin-3 and NT-pro BNP showed that the level of plasma galectin-3 was positively correlated with the level of plasma NT pro-BNP (r = 0.878, p < 0.001, Table 5).
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### Table 1. Plasma galectin-3 concentration in different clinical subgroups of HF.

<table>
<thead>
<tr>
<th>NYHA sub-groups</th>
<th>n</th>
<th>plasma galectin-3 (pg/mL)</th>
<th>Mean</th>
<th>Median</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I (mild HF)</td>
<td>16</td>
<td>88.6</td>
<td>82.7</td>
<td>64.5–112.7</td>
<td></td>
</tr>
<tr>
<td>Class II (mild HF)</td>
<td>28</td>
<td>253.9</td>
<td>267.2</td>
<td>214.3–293.5</td>
<td></td>
</tr>
<tr>
<td>Class III (moderate HF)</td>
<td>29</td>
<td>764.4</td>
<td>694.8</td>
<td>626.4–902.4</td>
<td></td>
</tr>
<tr>
<td>Class IV (severe HF)</td>
<td>27</td>
<td>1913.5</td>
<td>1530.4</td>
<td>1443.1–2384.0</td>
<td></td>
</tr>
</tbody>
</table>

Plasma galectin-3 concentration was significantly different between NYHA subgroups (p<0.05, one-way ANOVA).

### Table 2. P values by Bonferroni (multiple comparisons) test for comparison of plasma galectin-3 concentrations of the NYHA subgroups.

<table>
<thead>
<tr>
<th></th>
<th>Class II</th>
<th>Class III</th>
<th>Class IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Class II</td>
<td>-</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Class III</td>
<td>-</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 3. Calculation of proposed plasma galectin concentration (pg/mL) in different clinical subgroups (NYHA classes) of HF.

<table>
<thead>
<tr>
<th>NYHA classes</th>
<th>95%CI of plasma galectin-3 concentration</th>
<th>Bridging gaps between 95%CI of consecutive classes with half way of gaps on either side</th>
<th>Compromising class limits to nearest zero or five with cut-off value 100 pg/mL</th>
<th>Proposed plasma galectin-3 concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>64.5–112.7</td>
<td>64.5–163.5</td>
<td>100–165</td>
<td>100–460 (mild HF)</td>
</tr>
<tr>
<td>Class II</td>
<td>214.3–293.5</td>
<td>1635–459.95</td>
<td>165–460</td>
<td></td>
</tr>
<tr>
<td>Class III</td>
<td>626.4–902.4</td>
<td>459.95–1172.7</td>
<td>460–1170</td>
<td>460–1170 (moderate HF)</td>
</tr>
<tr>
<td>Class IV</td>
<td>1443.1–2384.0</td>
<td>1172.75–2384.0</td>
<td>&gt;1170</td>
<td>&gt;1170 (severe HF)</td>
</tr>
</tbody>
</table>

### Table 4. Comparisons of the level of NT-pro BNP and LV ejection fraction in NYHA functional class sub-groups.

<table>
<thead>
<tr>
<th>NYHA</th>
<th>LV EF- (%)</th>
<th>NT pro BNP (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>49.43±4.87</td>
<td>2140.3±2967.5</td>
</tr>
<tr>
<td>Class II</td>
<td>40.21±6.85</td>
<td>4043.4±4233.3</td>
</tr>
<tr>
<td>Class III</td>
<td>39.82±6.2</td>
<td>6163.9±8071.6</td>
</tr>
<tr>
<td>Class IV</td>
<td>31.82±6.8</td>
<td>6360.5±10180.0</td>
</tr>
</tbody>
</table>

### Table 5. Correlation analysis between the level of plasma galectin-3 and cardiac failure parameters (NT pro BNP and LVEF).

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>r</em></td>
</tr>
<tr>
<td>LV Ejection fraction (EF) - (%)</td>
<td>-0.634</td>
</tr>
<tr>
<td>NT pro BNP (pg/ml)</td>
<td>0.878</td>
</tr>
</tbody>
</table>

### Discussion

In this cross-sectional evaluation of 100 cases with HF, we reported a positive correlation between plasma galectin-3 concentration and clinical NYHA stage—i.e., as the clinical stage of disease increases, the galectin-3 level also increases. Thus, we suggest that plasma galectin-3 concentration can be used as a biomarker to aid clinical staging and severity assessment of HF.

Galectin-3 is an emerging marker of cardiac fibrosis, which is an important adverse predictor of risk in heart disease. Progressive cardiac congestion in HF generates mounting myocardial stress, resulting in galectin-3 synthesis from cardiac ventricles. The increasing myocardial stress parallels with the rising galectin-3 production. Therefore, galectin-3 appears to be positively correlated with the severity of HF, which could be exploited for therapeutic monitoring, clinical staging, risk assessment, and risk stratification of HF. Precise staging of HF is an important matter for planning the treatment, it has range from changing of life style to left ventricular assist devices implantation. Also planning medical therapy and choosing medication depends...
on staging.\textsuperscript{14} Approximately 50\% of deaths from heart failure (HF) are associated with arrhythmias as a result of electrolyte and acid-base abnormalities. Their incidence is mostly correlated with the severity of cardiac dysfunction.\textsuperscript{17}

Studies regarding the role of galectin-3 in therapeutic monitoring have already been published. It has been reported that there was no benefit in treating patients with HF NYHA II–IV with rosuvastatin when the galectin-3 concentration was high (>19 ng/mL), suggesting that the damage had already occurred.\textsuperscript{15} However, for patients with a low galectin-3 concentration (≤19 ng/mL), there was improvement in clinical outcomes with use of this statin.\textsuperscript{15} Galectin-3 monitoring may be particularly useful for HF treated with aldosterone antagonists because these drugs block cardiac fibrosis, which is the stimulus for galectin-3 release. For therapeutic monitoring, the galectin-3 assay has been approved in USA and Europe, and a test kit is commercially available.\textsuperscript{18–21}

Biomarkers such as galectin-3 that reflect ongoing remodeling via cardiac fibrosis of the heart may provide complementary information regarding the natriuretic peptides used in the management of chronic HF with regard to risk stratification for future adverse cardiac events (death, myocardial infarction, and need for heart transplantation).\textsuperscript{14} Plasma galectin-3 measurement is not very expensive, readily available, easily interpretable, and suitable for poor patients similar to those included in our study. The availability of this simple blood test could dramatically influence the landscape of HF management and its risk stratification. Accordingly, this will facilitate the rapid correct clinical staging of HF to reduce its morbidity and mortality. Galectin-3 has been considered to be a new promising biomarker for clinical staging of HF.\textsuperscript{7–11} However, in addition to the literature supporting the prognostic role of galectin-3, there is evidence against its use as a prognostic biomarker in HF.\textsuperscript{12–13} Therefore, the use of galectin-3 in clinical staging of HF requires more evidence.

In the present study, plasma galectin-3 concentration in clinical subgroups (NYHA class I–class IV) of cases with HF was found to be gradually increasing with the lowest concentration in class I and highest concentration in class IV, indicating an increasing plasma galectin-3 concentration with increasing severity of HF. This finding was consistent with previous studies. In the CORONA study, patients with HF who exhibited an increase in galectin-3 had the worst outcomes compared with those whose levels were stable or decreased, irrespective of the initial galectin level.\textsuperscript{15} Furthermore, the survival probability was <40\% in the highest galectin-3 quartile. In the same study, patients who had both a high galectin-3 concentration and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) had the worst outcomes.\textsuperscript{16}

To guide the clinical use of galectin-3 as a biomarker for clinical staging and to form a baseline for further studies, we proposed the range of plasma galectin-3 concentration for mild, moderate, and severe HF. We propose that the plasma galectin-3 concentration in mild, moderate, and severe HF is 100–460, 460–1170, and >1170 pg/mL, respectively. This is the first study proposing the galectin-3 levels for severity stages of HF.

In our study, the classification of heart failure with galectin-3 plasma levels was supported by EF and NT-pro BNP changes. Most studies reported using N-terminal pro-brain natriuretic peptide (NT-pro BNP) in diagnosis of heart failure but there is controversy about use of these tests in determining prognosis and classification of severity of heart failure. In addition that we found plasma NT-pro BNP levels positively correlated with increasing plasma galectin-3 levels and heart failure subgroups in our study. Furthermore, ejection fractions (EF) were found negatively correlated with galectin-3 plasma levels. That is a marker of validity of galectin-3 based heart failure classification.

The main limitation of our study was its cross-sectional design, which does not permit the evaluation of the galectin-3 concentration trend over time in different NYHA classes of HF. In addition, limited sample size precludes us from obtaining a definitive conclusion regarding the cutoff levels of galectin-3 for each clinical stage. Nevertheless, this study provides evidence regarding the possible use of plasma galectin-3 in clinical staging and thus management of HF.

**Conclusion**

In conclusion, plasma galectin-3 increases as severity or NYHA class of HF increases. Therefore, plasma galectin-3 can be used as a biomarker for clinical staging of HF. Further prospective, large-scale studies are required to confirm the range of galectin-3 concentration in patients with varying degrees of severity of HF.

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