Galectin-3 and N-Terminal of Prohormone Brain Natriuretic Peptide as Prognostic Biomarkers in Patients with Regression of Chronic Lymphocytic Leukemia

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Abstract

Aim: evaluate the prognostic value of galectin-3 (Gal-3) and N-terminal of prohormone brain natriuretic peptide (NT-proBNP) for cumulative survival in patients with regression of chronic lymphocytic leukemia.

Methods: One hundred fifty six subjects with regression of chronic lymphocytic leukemia. Observation period was up to 12 months. Blood samples for biomarkers measurements were collected. ELISA method for measurements of circulating level of Gal-3 was used. Concentrations of Gal-3 and NT-pro-brain natriuretic peptide (BNP) for cumulative survival cases due to advanced chronic heart failure (CHF) were tested.

Results: Two hundred sixteen cumulative clinical events occurred in 51 patients (32.7%) within the follow-up, with their distribution being as follows: 7 cardiovascular deaths, 122 cardiac arrhythmias, 16 cardiac ischemic events, 3 strokes, 30 chronic heart failures and 38 hospital admissions for cardiovascular
reasons. Medians of circulating levels of Gal-3 in subjects without and with cardiovascular events were 5.16 ng/ml (95% confidence interval [CI] = 4.74-5.56 ng/ml) and 16.4 ng/ml (95% CI = 14.80-18.01 ng/ml) (p<0.001) respectively. Medians of circulating levels of NT-proBNP in subjects without and with cardiovascular events were 13.14 fmol/ml (95% confidence interval [CI] = 10.94-15.35 fmol/ml) and 22.19 fmol/ml (95% CI = 12.00-33.38 fmol/ml) (p=0.07) respectively.

The results of regression analysis showed directly related circulating Gal-3 with \( \frac{E}{A_m} \) (\( r = 0.35 \), \( P = 0.002 \)), \( \frac{E}{E_m} \) (\( r = 0.35 \), \( P = 0.002 \)), NT-pro-BNP (\( r = 0.31 \), \( P = 0.017 \)), hypertension (\( r = 0.37 \), \( P = 0.001 \)), obesity (\( r = 0.41 \), \( P = 0.003 \)), T2DM (\( r = 0.39 \), \( P = 0.001 \)), TC (\( r = 0.34 \), \( P = 0.003 \)), LVEF (\( r = -0.38 \), \( P = 0.001 \)).

Gal-3, NT-pro-BNP, GFR, \( \frac{E}{E_m} \), LVEF, T2DM, hypertension, and multi-vessel lesion of coronary artery were selected as predictors in the univariate logistic regression analysis. Multivariate logistic regression revealed independent predictive value of circulating Gal-3 for one-year cumulative cardiovascular events (odds ratio [OR] = 1.13; 95% CI = 1.07–1.25; \( P = 0.003 \)). In fact, Gal-3, NT-pro-BNP, \( \frac{E}{E_m} \), and LVEF remained statistically significant predictors for cumulative cardiovascular events, whereas T2DM, hypertension, obesity, and multi-vessel lesion did not.

Conclusion: Increased circulating Gal-3 and NT-proBNP associate with increased one-year cumulative cardiovascular events among patients with documented chronic lymphocytic leukemia and known asymptomatic atherosclerosis.

**Keywords:** galectin-3; atherosclerosis; lymphoproliferative disease; survival; prognosis

**Introduction**

Nature development of chronic lymphocytic leukemia associates with increased risk of cardiovascular diseases and heart failure [1]. Activation of inflammatory cells, such as macrophages, due to lymphoproliferative diseases progression contributes plaque instability, vascular microcalcification, endothelial dysfunction, that are considered the pivotal mechanism of worsening vascular disease [10]. In fact, that some drugs (doxorubicin, cyclophosphamide) that used for treatment in chronic lymphocytic leukemia patients may induced endothelial dysfunction, discarded vascular repair mechanisms [7]. As cell-to-cell interactions are critical in the processes of lymphoproliferative diseases, galectin-3 (Gal-3) have become of interest as novel regulators of inflammation. Gal-3 is a member of a family of \( \beta \)-galactoside-binding lectins that recognize specific oligosaccharide, ligand glycoproteins or glycolipids on the membranes of neighboring cells or in the extracellular matrix [6]. Gal-3 is produced by activated macrophages and it is predominantly expressed in subclinical atherosclerosis, unstable and stable coronary artery disease, heart failure [3, 8, 9]. Therefore, Gal-3 is not only key player in inflammation and as well as in tumor progression by displaying
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intracellular and extracellular activities [5]. However, the predictive role of Gal-3 in stable patients with lymphoproliferative diseases is not understood.

European Guidelines emphasized the role of natriuretic peptides as potential markers for cardiovascular diseases and, especially, for heart failure [14]. Therefore, they seem to be independent mortality predictors in patients with chronic heart failure. Although most studies showed that NT-proBNP is a marker with a higher sensitivity and specificity; however the application of this analysis in clinical practice is often limited by the absence of a accepted range in patients with comorbidities including chronic lymphocytis leukemia.

The aim of the study to evaluate the prognostic value of galectin-3 and NT-proBNP for cumulative survival in subclinical atherosclerosis patients with controlled lymphoproliferative diseases.

Design and Methods

One hundred fifty six out subjects with full or partial remission of chronic lymphocytic leukemia who underwent cardiac computer tomography (CT) angiography and documented asymptomatic coronary artery disease (CAD) were enrolled in the study. All subjects gave their written informed consent to participation in the study. Diagnosis and staging of lymphoproliferative diseases were defined by current clinical practice guidelines [12, 13]. To be achieving remission chemotherapy with FC, R-FC, R-CHOP, CHOP, mini-CHOP, COP, was used accordingly contemporary clinical guidelines. All subjects were at full or partial remission stage at baseline.

Echocardiography in B-mode was performed accordingly to Recommendation of American Society of Echocardiography on the scanner “MyLab 50” (Italy) using a transducer with a frequency of 2.5-3.5 MHz. End-diastolic and end-systolic LV volumes were obtained using a two-dimensional reference sector according to Simpson’s method, and LV ejection fraction (LVEF) was calculated by accordingly conventional methods [4].

Coronary vessel-wall, and plaque geometrical, and compositional parameters were measured on contrast-enhanced spiral computer tomography (CT) angiography [2]. Contrast-enhanced spiral CT was performed on the “OPTIMA CT 660” scanner (GE Medical Systems, and Milwaukee, USA) with 2 rows of detectors (32 × 2 CT system) during the end-expiratory breath-hold. After noncontrast localization image acquisition, injection of nonionic contrast “Omnipak” (Amersham Health, Ireland) was used to determine the optimal coronary arterial image. Standardized calcium scores were obtained with beam energy of 120 kV, full rotation time of 350 milliseconds, and tube current of 300 mA. The images were reconstructed in 0.6-mm axial slices. Scans were electrocardiogram-gated and were triggered at either 40% or 75% phase contingent on heart rate.

All blood samples were collected after fasting in cooling vacutaner and after that it was immediately centrifugated (4°C for 6.000 × 15 min). After centrifugation serum was blind coded and stored at -70° until used. Human Gal-3
and NT-proBNP were measured by ELISA technique (ELISA kits manufactured by R&G, United Kingdom) used for examination. All determinations were done by duplicating. Fasting plasma glucose (FPG) was quantified by the glucose oxidase procedure; HbA1c was measured by ion-exchange high-performance liquid chromatography (HPLC; Bio-Rad, Hercules, CA, USA).

Concentrations of total cholesterol (TC) and high density lipoprotein (HDL) cholesterol were determined by a Dimension Clinical Chemistry System (Dade Behring Inc, Newark, NJ). Low density lipoprotein (LDL) cholesterol was calculated by using the formula of Friedewald W.T., Levy R.I., Fredrickson D.S. (1972). All measurements and blood sample for were collected at the same visit.

Clinical Events: Screening and Diagnostics

Clinical interviews were carried out every month for three years after baseline. The following are the clinical events verified: newly diagnosed strokes or TIAS; death for any reasons and sudden cardiac death; coronary ischemic events (myocardial infarction, unstable angina) that needed hospital admission for cardiovascular reasons, new-onset chronic heart failure. Newly diagnosed strokes were confirmed with CT. All clinical events were presented as cumulative.

Statistical Analysis

All statistical analyses were performed in SPSS for Windows v. 17.0 (SPSS Inc., Chicago, IL, USA). All values were given as mean and 95% CI or median and percentiles. An independent group t-test was used for comparisons for all interval parameters meeting the criteria of normality and homogeneity of variance. For interval parameters not meeting these criteria, the non-paramentric Mann-Whitney test was used to make comparisons between the groups. Comparisons of categorical variables between the groups were performed using the Chi2 test, and the Fisher exact test. The potential factors that may be associated with cardiovascular events was identified first by the univariate analysis, then multivariate logistic regression analyses were used to identify the predict factors. A calculated difference of P<0.05 was considered significant.

Results

Two hundred sixteen cumulative clinical events occurred in 51 patients (32.7%) within the follow-up, with their distribution being as follows: 7 deaths, 122 cardiac arrhythmias, 16 cardiac ischemic events, 3 strokes (2 lacunar infarctions and 1 cardioembolic strokes), 30 CHF and 38 hospital admissions for cardiovascular reasons.

General characteristics of study patients are presented in table 1. The patients of both cohorts were age-, sex-, conventional risk factor-, hemodynamic parameters matched. There was significant difference between both cohorts in incidences of heart failure (p<0.001). Therefore, circulating level of NT-pro-BNP
was lower in free-events subject cohort when compared with subjects cohort with cardiovascular events occurred.

Medians of circulating levels of NT-pro-BNP in subjects without and with cardiovascular events were 13.14 fmol/ml (95% confidence interval [CI] = 10.94-15.35 fmol/ml) and 22.19 fmol/ml (95% CI = 12.00-32.38 fmol/ml) (p=0.07) respectively. Medians of circulating levels of NT-pro-BNP in subjects without and with heart failure were 13.47 fmol/ml (95% confidence interval [CI] = 11.44-15.51 fmol/ml) and 37.64 fmol/ml (95% CI = 7.51-67.77 fmol/ml) (p<0.001) respectively. (Figure 1).

![Figure 1: Boxplot shows a significant difference between medians of circulating NT-pro-BNP in patients without (1) and with (2) heart failure.](image)

All hypertensive patients were treated according to current clinical guidelines with diet, lifestyle modification and drug therapy that included ACE inhibitors / ARBs, aspirin or other antiagregants, statins. Metformin was prescribed in 3 (2.9%) and 3 (5.9) T2DM patients in both cohorts, in other cases diet and life-style modification were recommended only. Because incidences of CHF was seen significantly often in subjects cohort with cardiovascular events in comparison with free-events subject cohort, however, ACEI / ARAs, mineralcorticoid receptor antagonists, and diuretics have been used frequent.

Medians of circulating levels of Gal-3 in subjects without and with cardiovascular events were 5.16 ng/ml (95% confidence interval [CI] = 4.74-5.56 ng/ml) and 16.4 ng/ml (95% CI = 14.80-18.01 ng/ml) (p<0.001) respectively (Figure 2).
Figure 2: Boxplot shows a significant difference between medians of circulating Gal-3 in patients without (1) and with (2) cardiovascular events.

Medians of circulating levels of Gal-3 in subjects without and with heart failure were 7.49 ng/ml (95% confidence interval [CI] = 6.28-8.70 ng/ml) and 18.28 ng/ml (95% CI = 17.42-19.13 ng/ml) (p<0.001) respectively (Figure 3).

The results of regression analysis showed directly related circulating Gal-3 with NT-pro-BNP (r = 0.31, p = 0.017), E/Em (r = 0.35, p = 0.002), hypertension (r = 0.37, p = 0.001), obesity (r = 0.41, p = 0.001), T2DM (r = 0.39, p = 0.001), TC (r = 0.34, p = 0.003), LVEF (r = -0.38, p = 0.001).

Gal-3, NT-pro-BNP, GFR, E/Em, LVEF, T2DM, hypertension, and multi-vessel lesion of coronary artery were selected as predictors in the univariate logistic regression analysis (Table 2).
Multivariate logistic regression revealed independent predictive value of circulating Gal-3 for one-year cumulative cardiovascular events (odds ratio [OR] = 1.13; 95% CI = 1.07–1.25; p = 0.003).

Multivariate logistic regression revealed independent predictive value of circulating NT-pro-BNP for one-year cumulative cardiovascular events (odds ratio [OR] = 1.05; 95% CI = 1.02–1.14; p = 0.001).

In fact, Gal-3, NT-pro-BNP, E/Em, and LVEF remained statistically significant predictors for cumulative cardiovascular events, whereas T2DM, hypertension, obesity, and multi-vessel lesion did not.

Table 1 General characteristic of patients participating in the study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Free-events subjects (n=105)</th>
<th>Subjects with cardiovascular events (n=51)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>60,79±9,26</td>
<td>62,02±8,48</td>
<td>0,40</td>
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<tr>
<td>Males, n (%)</td>
<td>55 (52,4)</td>
<td>31 (60,8)</td>
<td>0,34</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>14 (13,3)</td>
<td>11 (10,8)</td>
<td>0,19</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>27 (25,7)</td>
<td>19 (37,6)</td>
<td>0,44</td>
</tr>
<tr>
<td>T2DM, n (%)</td>
<td>3 (2,9)</td>
<td>3 (5,9)</td>
<td>0,62</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27,15±3,49</td>
<td>27,48±3,51</td>
<td>0,83</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>11 (10,5)</td>
<td>10 (19,6)</td>
<td>0,81</td>
</tr>
<tr>
<td>Overweight, n (%)</td>
<td>38 (36,1,6)</td>
<td>10 (19,6)</td>
<td>0,45</td>
</tr>
<tr>
<td>Adherence to smoking, n (%)</td>
<td>6 (5,7)</td>
<td>6 (11,8)</td>
<td>0,41</td>
</tr>
<tr>
<td>GFR, mL/min/1.73 m²</td>
<td>102,09±19,28</td>
<td>107,08±15,93</td>
<td>0,07</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5,27±0,74</td>
<td>5,41±0,68</td>
<td>0,32</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>4,69±0,60</td>
<td>4,71±0,65</td>
<td>0,79</td>
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<tr>
<td>Creatinine, μmol/L</td>
<td>69,04±13,88</td>
<td>67,66±11,88</td>
<td>0,78</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4,89±0,72</td>
<td>4,91±0,92</td>
<td>0,96</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3,06±0,79</td>
<td>3,26±0,88</td>
<td>0,25</td>
</tr>
</tbody>
</table>
Table 1 (Continued): General characteristic of patients participating in the study

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=100)</th>
<th>Group 2 (n=200)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.35±0.37</td>
<td>1.29±0.41</td>
<td>0.38</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>118.59±15.61</td>
<td>120.41±18.67</td>
<td>0.52</td>
</tr>
<tr>
<td>Heart rate, beats per 1 min.</td>
<td>80.48±9.53</td>
<td>78.08±10.82</td>
<td>0.28</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>56.83±5.63</td>
<td>54.36±3.55</td>
<td>0.71</td>
</tr>
<tr>
<td>E/Am, U</td>
<td>1.02±0.14</td>
<td>1.00±0.21</td>
<td>0.14</td>
</tr>
<tr>
<td>E/Em, U</td>
<td>7.02±1.59</td>
<td>8.72±2.60</td>
<td>0.11</td>
</tr>
<tr>
<td>ACEI or ARAs, n (%)</td>
<td>9 (8.6)</td>
<td>12 (23.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acetylsalicylic acid, n (%)</td>
<td>85 (80.9)</td>
<td>47 (92.1)</td>
<td>0.21</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>55 (52.4)</td>
<td>15 (29.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Metformin, n (%)</td>
<td>3 (2.9)</td>
<td>3 (5.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Loop diuretics, n (%)</td>
<td>3 (2.9)</td>
<td>10 (19.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mineralcorticoid receptor antagonists, n (%)</td>
<td>2 (1.9)</td>
<td>6 (11.8)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: * - statistically differences between parameters in the two groups (P<0.05); CI – confidence interval; CAD – coronary artery disease, T2DM – type two diabetes mellitus, GFR – Glomerular filtration rate, HDL-C – high-density lipoprotein cholesterol, LDL-C – Low-density lipoprotein cholesterol, BP – blood pressure, BMI – Body mass index, BNP – brain natriuretic peptide, LVEF - Left ventricular ejection fraction, U – unit, Em – early diastolic myocardial velocity, Am – late diastolic myocardial velocity, E – peak velocity of early diastolic left ventricular filling, ACEI – angiotensin-converting enzyme inhibitor, ARAs – angiotensin-2 receptors antagonists.

Discussion

In this study in subjects with established chronic lymphocytic leukemia, traditional risk markers had little predictive value for recurrent cardiovascular events and cardiovascular mortality. Of all biomarkers NT-proBNP was by far the strongest, adding substantial predictive value beyond the traditional risk markers.

Gal-3 is a unique chimera-type member of the β-galactoside-binding soluble lectin family involved in several biological functions such as intracellular signaling, cell to cell interaction and exchanges between cells and the extracellular
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matrix. It is well known that Gal-3 is therefore participating to the control of gene expression, the regulation of immune response and the control of cell growth and viability [6]. Recent researches suggest that Gal-3 plays various roles in pathogenesis of cardiovascular diseases and malignancy [3, 9, 11]. Gal-3 is also involved in immune-mediated cell damage through inducing cell apoptosis, increasing both IL-17 and IFN-gamma synthesis, but decreasing IL-10 production [11]. All these mechanisms are not unique and are involved in plaque instability.

Results of the study showed that exaggerated circulating level of Gal-3 in stable patients with chronic lymphocytic leukemia may consider biomarker with power predictive value for cardiovascular events whether for tumor progression did not. Probably it may be related with small size of the study or short-term period of the observation. However, association of Gal-3 with tumor progression was not found. Author think that it is needed more studies with higher statistical power to be recognized prognostic potential of Gal-3 in two directions: cardiovascular outcomes and tumor progression. Taken together, it could be discussed around cut-off of Gal-3 plasma level that is suitable for stable and unstable patients with chronic lymphocytic leukemia associated with different risk of tumor progression.

In conclusion, it is found that increased circulating Gal-3 and NT-proBNP associate with increased one-year cumulative cardiovascular events among patients with documented chronic lymphocytic leukemia and known asymptomatic coronary atherosclerosis.

Conflict of interests: not declared

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Limitations of the study. This study has some limitations. We believed that a greater cohort would be desirable to improve the power of the study. We also relied on clinical data to rule out infection and other inflammatory diseases before sampling, but we couldn’t exclude that some patients had unrecognized the conditions responsible for the elevated Gal-3 levels.

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http://dx.doi.org/10.1002/1097-0142(19800915)46:6<1479::aid-cncc2820460631>3.0.co;2-r

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