Galectin-3 as a Prognostic Biomarker in Subclinical Atherosclerosis Patients with Lymphoproliferative Diseases

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Abstract

Aim: evaluate the prognostic value of galectin-3 (Gal-3) for cumulative survival in subclinical atherosclerosis patients with controlled lymphoproliferative diseases.

Methods: One hundred eighty two out subjects with full or partial remission of lymphoproliferative diseases (43 patients with multiple myeloma, 101 subjects with chronic lymphoid leukemia, and 10 patients with Hodgkin's and non-Hodgkin's lymphoma. Observation period was up to 3 years. 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 CHF were tested.

Results: One hundred ninety six cumulative clinical events occurred in 42 patients (23.1%) within the follow-up, with their distribution being as follows: 29 deaths, 65 cardiac arrhythmias, 17 cardiac ischemic events, 9 strokes, 37 chronic heart failures and 39 hospital admissions for cardiovascular reasons. Medians of circulat-

ing levels of Gal-3 in subjects without and with cardiovascular events were 5.10 ng/ml (95% confidence interval [CI] = 4.70-5.50 ng/ml) and 16.5 ng/ml (95% CI = 14.80-18.10 ng/ml) ($P < 0.001$) respectively. The results of regression analysis showed directly related circulating Gal-3 with E/Am ($r = 0.44$, $P = 0.001$), E/Em ($r = 0.42$, $P = 0.001$), NT-pro-BNP ($r = 0.43$, $P = 0.002$), hypertension ($r = 0.42$, $P = 0.001$), obesity ($r = 0.40$, $P = 0.003$), T2DM ($r = 0.41$, $P = 0.001$), multi-vessel lesion of coronary arteries ($r = 0.36$, $P = 0.001$), gender ($r = 0.32$, $P = 0.001$ for male), TC ($r = 0.32$, $P = 0.001$), GFR ($r = -0.36$, $P = 0.001$), LVEF ($r = -0.32$, $P = 0.001$).

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. Multivariate logistic regression revealed independent predictive value of circulating Gal-3 for three-year cumulative cardiovascular events (odds ratio [OR] = 1.12; 95% CI = 1.06–1.27; $P = 0.003$). In fact, Gal-3, NT-pro-BNP, GFR, 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 associates with increased 3-year cumulative cardiovascular events among patients with documented lymphoproliferative diseases and known asymptomatic atherosclerosis.

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

Introduction

Nature development of lymphoma and multiple myeloma associates with increased risk of cardiovascular diseases and atherothrombotic events [8]. 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 (bendamustin, cladribin) that used for treatment in lymphoma and multiple myeloma patients may induced endothelial dysfunction and discorded vascular repair mechanisms [6]. 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 β -galactoside-binding lectins that recognize specific oligosaccharide, ligand glycoproteins or glycolipids on the membranes of neighboring cells or in the extracellular matrix [5]. Gal-3 is produced by activated macrophages and it is predominantly expressed in subclinical atherosclerosis, unstable and stable coronary artery disease, heart failure [2, 7, 9]. Therefore, Gal-3 is not only key player in inflammation and as well as in tumor progression by displaying intracellular and

extracellular activities [4]. However, the predictive role of Gal-3 in stable patients with lymphoproliferative diseases is not understood. The aim of the study to evaluate the prognostic value of galectin-3 for cumulative survival in subclinical atherosclerosis patients with controlled lymphoproliferative diseases.

Design and Methods

One hundred eighty two out subjects with full or partial remission of lymphoproliferative diseases (43 patients with multiple myeloma, 101 subjects with chronic lymphoid leukemia, and 10 patients with Hodgkin's and non-Hodgkin's lymphoma) 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, ABVD, VTD, CTD, TD, bendamustine, cladribine 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 [3].

Coronary vessel-wall, and plaque geometrical, and compositional parameters were measured on contrast-enhanced spiral computer tomography (CT) angiography [1]. 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 vacutainer 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-pro-brain natriuretic peptide (BNP) 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-parametric 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

One hundred ninety six cumulative clinical events occurred in 42 patients (23.1%) within the follow-up, with their distribution being as follows: 29 deaths, 65 cardiac arrhythmias, 17 cardiac ischemic events, 9 strokes (5 lacunar infarctions and 2 cardioembolic strokes), 37 CHF and 39 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 higher in free-events subject cohort when compared with subjects cohort with cardiovascular events occurred.

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 (2.1%) and 5 (11.9) 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 5.10 ng/ml (95% confidence interval [CI] = 4.70-5.50 ng/ml) and 16.5 ng/ml (95% CI = 14.80-18.10 ng/ml) ($P < 0.001$) respectively (Figure 1).

Figure 1: Boxplot shows a significant difference between medians of circulating Gal-3 in died and survived patients

The results of regression analysis showed directly related circulating Gal-3 with E/Am ($r = 0.44$, $P = 0.001$), E/Em ($r = 0.42$, $P = 0.001$), NT-pro-BNP ($r = 0.43$, $P = 0.002$), hypertension ($r = 0.42$, $P = 0.001$), obesity ($r = 0.40$, $P = 0.003$), T2DM ($r = 0.41$, $P = 0.001$), multi-vessel lesion of coronary arteries ($r = 0.36$, $P = 0.001$), gender ($r = 0.32$, $P = 0.001$ for male), TC ($r = 0.32$, $P = 0.001$), GFR ($r = -0.36$, $P = 0.001$), LVEF ($r = -0.32$, $P = 0.001$).

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 three-year cumulative cardiovascular events (odds ratio [OR] = 1.12; 95% CI = 1.06–1.27; $P = 0.003$). In fact, Gal-3, NT-pro-BNP, GFR, 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

Variables	Free-events subjects (n=140)	Subjects with cardiovascular events (n=42)	P-value
Age, years	59±11.47	61±9.73	0.56
Males, n (%)	73 (52.1)	29 (69.0)	0.21
Hypertension, n (%)	15 (10.7)	5 (11.9)	0.28
Dyslipidemia, n (%)	28 (20.0)	11 (26.2)	0.36
T2DM, n (%)	17 (12.1)	5 (11.9)	0.56
BMI, kg/m ²	26.5 (95% CI=25.95-27.05)	28.2 (95% CI=27.02-28.38)	0.42
Obesity, n (%)	32 (23.6)	10 (23.8)	0.82
Overweight, n (%)	20 (14.3)	5 (11.9)	0.52
Adherence to smoking, n (%)	3 (2.1)	8 (1.9)	0.44
Heart failure, n (%)	9 (6.4)	18 (42.9)	<0.001
GFR, mL/min/1.73 m ²	103.9±19.4	105.5±14.4	0.48
HbA1c, %	5.22±0.7	5.42±0.83	0.58
Fasting blood glucose, mmol/L	4.63±0.57	4.71±0.67	0.69
Creatinine, µmol/L	68.88±13.78	73.02±12.96	0.72
Total cholesterol, mmol/L	4.94±0.75	5.0±0.75	0.56
LDL-C, mmol/L	2.79±0.72	3.0±0.74	0.42
HDL-C, mmol/L	1.52±0.33	1.46±0.38	0.44
NT-pro-BNP, pg/mL	9.54±18.94	35.99±45.85	0.062
Systolic BP, mm Hg	120.9±24.59	123.75±12.79	0.64
Heart rate, beats per 1 min.	79.4±11.67	74.66±4.64	0.62
LVEF, %	56.12±3.18	53.8±4.34	0.54
E/Am, U	1.11±0.17	0.98±0.21	0.12
E/Em, U	7.9±1.98	9.2±2.12	0.16
ACEI or ARAs, n (%)	15 (10.7)	18 (42.9)	<0.001
Mineralcorticoid receptor antagonists, n (%)	9 (6.4)	15 (35.7)	0.001
Acetylsalicylic acid, n (%)	124 (88.5)	40 (95.2)	0.16
Statins, n (%)	83 (59.3)	11 (28.6)	<0.01
Metformin, n (%)	3 (2.1)	5 (11.9)	0.001
Loop diuretics, n (%)	3 (2.1)	23 (54.8)	0.001

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.

Table 2: Univariate and multivariate predictors of three-year cumulative cardiovascular events

Variables	Univariate OR (95% CI)	Wald χ^2	P-value	Multivariate OR (95% CI)	Wald χ^2	P-value
Gal-3	1.16 (1.08–1.43)	11.7	0.009	1.12 (1.06–1.27)	10.2	0.003
GFR	1.10 (1.02–1.13)	8.5	0.001	1.05 (1.02–1.09)	8.1	0.001
LVEF	1.12 (1.08–1.14)	6.2	0.001	1.08 (1.04–1.12)	7.0	0.003
E/Em	1.06 (1.04–1.10)	7.2	0.003	1.01 (0.87–1.03)	5.8	0.004
E/Am	1.03 (1.01–1.05)	6.6	0.002	0.97 (0.88–1.05)	5.1	0.003
NT-pro-BNP	1.15 (1.08–3.10)	8.7	0.001	1.12 (1.04–2.17)	7.1	0.001
T2DM	1.08 (1.02–1.14)	2.2	0.001	1.00 (0.90–1.06)	2.01	0.001
Obesity	0.96 (0.88–1.07)	1.10	0.002	0.92 (0.90–1.02)	1.02	0.001
Hypertension	1.03 (1.01–1.05)	0.27	0.001	0.98 (0.92–1.06)	0.22	0.001
Multi-vessel lesion of coronary artery	1.02 (1.00–1.07)	0.21	0.001			

Discussion

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 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 [5]. Recent researches suggest that Gal-3 plays various roles in pathogenesis of cardiovascular diseases and malignancy [2, 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 this 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 lymphoproliferative diseases 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 lymphoproliferative diseases associated with different risk of tumor progression.

In conclusion, it is found that increased circulating Gal-3 associates with increased 3-year cumulative cardiovascular events among patients with documented lymphoproliferative diseases 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.

References

- [1] D.A. Bluemke, S. Achenbach, M. Budoff, T.C. Gerber, B. Gersh, L.D. Hillis, W.G. Hundley, W.J. Manning, B.F. Printz, M. Stuber, P.K. Woodard, Noninvasive coronary artery imaging: magnetic resonance angiography and multidetector computed tomography angiography: a scientific statement from the American Heart Association Committee on Cardiovascular Imaging and Intervention of the Council on Cardiovascular Radiology and Intervention, and the Councils on Clinical Cardiology and Cardiovascular Disease in the Young, *Circulation*, **118** (2008), 586 - 606.
- [2] C. Falcone, S. Lucibello, I. Mazzucchelli, S. Bozzini, A. D'Angelo, S. Schirinzi, R. Totaro, R. Falcone, M. Bondesan, G. Pelissero, Galectin-3 plasma levels and coronary artery disease: a new possible biomarker of acute coronary syndrome, *Int. J. Immunopathol. Pharmacol.*, **24** (2011), 905 - 913.
- [3] J.M. Gardin, D.B. Adams, P.S. Douglas, H. Feigenbaum, D.H. Forst, A.G. Fraser, P.A. Grayburn, A.S. Katz, A.M. Keller, R.E. Kerber, B.K. Khandheria, A.L. Klein, R.M. Lang, L.A. Pierard, M.A. Quinones, I. Schnittger, American Society of Echocardiography. Recommendations for a standardized report for adult transthoracic echocardiography: a report from the American Society of Echocardiography's Nomenclature and Standards Committee and Task Force for a Standardized Echocardiography Report, *J. Am. Soc. Echocardiogr.*, **15** (2002), 275 - 290.
- [4] M. Giordano, D.O. Croci, G.A. Rabinovich, Galectins in hematological malignancies, *Curr. Opin. Hematol.*, **20** (2013), 327 - 335.
- [5] D. Gruson, G. Ko, Galectins testing: new promises for the diagnosis and risk stratification of chronic diseases?, *Clin. Biochem.*, **45** (2012), 719 - 726.
- [6] T. Ishida, Triplets therapy for the patients of multiple myeloma, *Nihon Rinsho*, **9** (2013), 1691 - 1696.
- [7] P. Jarolim, Overview of cardiac markers in heart disease, *Clin. Lab. Med.*, **34** (2014), 1 - 14.
- [8] J. Laubach, T. Hideshima, P. Richardson, K. Anderson, Clinical translation in multiple myeloma: from bench to bedside, *Semin. Oncol.*, **5** (2013), 549 - 553.

- [9] W.C. Meijers, A.R. van der Velde, R.A. de Boer, The ARCHITECT galectin-3 assay: comparison with other automated and manual assays for the measurement of circulating galectin-3 levels in heart failure, *Expert. Rev. Mol. Diagn.*, **14** (2014), 257 - 266.
- [10] S. Menini, C. Iacobini, C. Ricci, C. Blasetti Fantauzzi, L. Salvi, C.M. Pesce, M. Relucenti, G. Familiari, M. Taurino, G. Pugliese, The galectin-3/RAGE dyad modulates vascular osteogenesis in atherosclerosis, *Cardiovasc. Res.*, **100** (2013), 472 - 480.
- [11] G. Radosavljevic, V. Volarevic, I. Jovanovic, M. Milovanovic, N. Pejnovic, N. Arsenijevic, D.K. Hsu, M.L. Lukic, The roles of Galectin-3 in autoimmunity and tumor progression, *Immunol. Res.*, **52** (2012), 100-110.
- [12] E. Terpos, G. Morgan, M.A. Dimopoulos, M.T. Drake, S. Lentzsch, N. Raje, O. Sezer, R. García-Sanz, K. Shimizu, I. Turesson, T. Reiman, A. Jurczyszyn, G. Merlini, A. Spencer, X. Leleu, M. Cavo, N. Munshi, S.V. Rajkumar, B.G. Durie, G.D. Roodman, International Myeloma Working Group recommendations for the treatment of multiple myeloma-related bone disease, *J. Clin. Oncol.*, **18** (2013), 2347 - 2357.
- [13] X.G. Zhou, Towards standardization of lymphoma diagnosis, *Zhonghua Bing Li Xue Za Zhi*, **4** (2013), 220 - 221.

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