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Galectin-3 is an independent predictor of survival in systemic sclerosis

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ABSTRACT

Background: Galectin-3 is a beta-galactoside-binding lectin that may be related to tissue sclerosis or aberrant activation of angiogenesis in systemic sclerosis (SSc). The aim of our study was to determine the associations between galectin-3 levels and patient characteristics, as well as to investigate the long term prognostic value of galectin-3 in a large cohort of SSc patients.
Methods: 152 patients with SSc (55 ± 11 years, 138 female) were included in our follow-up study. Blood samples and clinical data were collected at baseline. Primary and secondary outcomes were all-cause and cardiovascular mortality, respectively.
Resultss: Galectin-3 levels showed positive correlation with the grade of left ventricular diastolic function ($r = 0.193$; $p = 0.026$), erythrocyte sedimentation rate ($r = 0.172$; $p = 0.036$) and serum level of C-reactive protein ($r = 0.200$; $p = 0.015$) while negative correlation with diffusing capacity for carbon monoxide ($r = -0.228$; $p = 0.006$), in age, gender and BSA adjusted analyses. During the follow-up of 7.2 ± 2.3 years, 35 SSc patients (23%) died. In multivariate Cox regression analyses adjusted for age, gender, BSA, creatinine and NT-proBNP levels, galectin-3 was an independent predictor both of the all-cause mortality (HR: 2.780, 95% CI: 1.320–5.858, $p = 0.007$) and cardiovascular mortality (HR: 3.346, 95% CI: 1.118–10.012, $p = 0.031$). Using receiver-operating characteristic analysis, galectin-3 > 10.25 ng/ml was found to be the best predictor of the all-cause mortality.
Conclusions: Our results suggest that galectin-3 is an independent predictor of all-cause and cardiovascular mortality in SSc. Validation studies are required to establish whether galectin-3 may be proposed as simple biomarker for identifying patients with high mortality risk in SSc.

1. Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by vasculopathy and progressive fibrosis of the skin and certain internal organs. Although the pathogenesis of the disease is still not fully understood, it is known, that activated fibroblasts are responsible for the development of fibrosis and accumulation of extracellular matrix molecules leading to internal organ dysfunction. In addition, there are several growth factors, chemokines, and cytokines associated with these processes [1,2].

Galectin-3 is a beta-galactoside-binding member of the lectin family that plays an important role in more biologic processes, including cell proliferation, adhesion, differentiation and apoptosis. Evidences indicate that galectin-3 activates a variety of profibrotic factors, promotes fibroblast proliferation and transformation, and mediates collagen production [3]. Another important aspect of galectin-3 is to exert a potent proangiogenic effect in accordance with other proangiogenic factors, such as vascular endothelial growth factor and basic fibroblast growth factor [4]. Recent clinical studies suggest that galectin-3 may be related to the developmental process of skin and organ sclerosis as well as to the aberrant activation of angiogenesis in SSc, but the available data are inconsistent [3,5,6].

Therefore, the aim of our study was to assess the potential associations between serum galectin-3 levels and disease characteristics in a large cohort of SSc patients, as well as to investigate the long term prognostic value of galectin-3 in these patients. Since the diagnostic and

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prognostic value of NT-proBNP is well established in SSc [7–10], this biomarker served as a benchmark in our investigation.

2. Methods

2.1. Study population

Patients diagnosed with SSc in the tertiary centre of the Department of Rheumatology and Immunology, University of Pécs were recruited into our prospective study. They were required to fulfil the American College of Rheumatology criteria for SSc and were classified as having limited cutaneous or diffuse cutaneous SSc according to the criteria described by LeRoy et al. [11]. All enrolled cases complied with the recently updated ACR/EULAR classification criteria [12]. Blood samples of consecutive patients were collected for storage between 1st January 2005 and 31st December 2008. Baseline clinical (body surface area-BSA, Rodnan skin score), laboratory (erythrocyte sedimentation rate, C-reactive protein-CRP, creatinine, hemoglobin), spirometric (forced vital capacity-FVC; diffusing capacity for carbon monoxide-DLCO) and echocardiographic (ejection fraction, calculated right ventricular systolic pressure) data were collected at the same time. Characterization of left ventricular diastolic function was based on mitral inflow pattern (E/A ratio), left ventricular wall thickness and left atrial size [13]. Duration of the disease was defined as time between the onset of the first non-Raynaud symptom of SSc and the inclusion, in years.

2.2. Follow-up

Follow-up time was defined as the time between the date of blood sample collection and the date of death or the last clinical visit.

During clinical visits detailed medical history was obtained from all subjects. Significant coronary artery disease was defined as coronary artery stenosis >50% proved by invasive measurements or as history of previous myocardial infarction. The diagnosis of pulmonary arterial hypertension (PAH) was based on results obtained by right heart catheterization (mean pulmonary artery pressure ≥ 25 mmHg and pulmonary capillary wedge pressure ≤ 15 mmHg). Patients with both transient and chronic atrial fibrillation were recorded. Severe pulmonary involvement was diagnosed when diffuse fibrosis or honeycombing was detected by high resolution CT and FVC <50% was measured by spirometry.

Medical records of the deceased patients were collected and treating physicians and relatives were interviewed. Data obtained from these sources were reviewed by a team of rheumatologists and cardiologists to determine the single leading cause of death. As the cases with considerable overlap between causes of death were relatively common, all-cause mortality was chosen as primary outcome. As secondary endpoint, cardiovascular mortality was also investigated.

The study complied with the Declaration of Helsinki. The institutional ethics committee approved the study. All subjects had given written informed consent prior to blood sample collection and storage.

2.3. Galectin-3 and NT-proBNP assays

Blood collection for galectin-3 and NT-proBNP assessments was performed at baseline. Frozen samples was stored at −80 °C and thawed prior to testing. Analysis of galectin-3 levels was performed using Human Galectin-3 Platinum ELISA kit developed by eBioscience (San Diego, CA, USA). NT-proBNP was measured using electrochemiluminescence immunoassay on the Elecsys 2010 system (Roche Diagnostics, Mannheim, Germany).

2.4. Statistical analysis

Categorical data were expressed as frequencies and percentages; continuous data were expressed as mean ± SD. Comparisons between groups were performed using independent samples *t*-tests or Mann-Whitney test for continuous variables while chi square test for categorical variables.

Clinical variables that correlate with galectin-3 level were determined using bivariate Pearson correlation. In a second step, partial correlation method was used to correct the results for age, gender and BSA. Since concentration of galectin-3 and NT-proBNP did not show normal distribution, logarithmic transformation was performed.

Relationship between logarithmic transformed galectin-3 and mortality was investigated by using Cox proportional hazards models (backward stepwise), including adjustment for age, gender, BSA and creatinine. Finally, lnNT-proBNP was also added to the model, given its known association with outcomes in systemic sclerosis. Hazard ratios (HR) were calculated with 95% confidence intervals (CI).

Receiver-operating characteristic (ROC) curves were used to examine the performance of galectin-3 and NT-proBNP in predicting all-cause mortality. Area under the curve (AUC) was calculated. Optimal cut-off value was chosen to maximize sensitivity and specificity. Based on these cut-off values, Kaplan-Meier survival curves were created and differences between groups were tested by Mantel-Cox log rank test.

Prognostic power of concordant versus discordant values for NT-proBNP and galectin-3 was also evaluated. For this purpose, four groups were created defined by dividing each variable at the cut-off value obtained by ROC curve previously (low galectin-3/low NT-proBNP, high galectin-3/low NT-proBNP, low galectin-3/high NT-proBNP, high galectin-3/high NT-proBNP). Kaplan-Meier survival curve was created and differences between groups were tested by Mantel-Cox log rank test.

Additional prognostic value of the inclusion of elevated galectin-3 level as a new parameter to risk prediction models were tested including changes in model fit assessed by the χ^2 Omnibus test, area under curve in ROC analysis and reclassification including elevated galectin-3 (detailed description of the method is presented in the Online Supplement).

Level of significance was set at $p < 0.05$. Calculations were performed with IBM SPSS 22 statistical software.

3. Results

152 SSc patients were enrolled into the study. Mean age of the study cohort was 55 years. 107 patients had limited cutaneous while 45 patients had diffuse cutaneous form of the disease. Preserved (≥55%), moderately reduced (35–54%) and severely reduced (<35%) EF was found in 138 (90.8%), 13 (8.6%) and 1 (0.6%) patients, respectively. Baseline clinical data of the 152 SSc patients as well as detailed description of the organ involvement, co-morbidities and medication are outlined in Table 1.

3.1. Correlations of galectin-3 and NT-proBNP with clinical variables

Both biomarkers showed positive correlation with age. Galectin-3 levels showed positive correlation with the duration of the SSc, but this correlation lost its significance in the age, gender and BSA adjusted analysis. NT-proBNP, but not galectin-3 levels significantly correlated with right ventricular systolic pressure and with the diagnosis of PAH. Negative correlation was found between DLCO and both biomarkers (Fig. 1A). Both biomarkers correlated positively with the grade of left ventricular diastolic dysfunction (Fig. 1B) as well as with the laboratory parameters of inflammation. Further correlations of galectin-3 and NT-proBNP with clinical variables are reported in Table 2.

3.2. All-cause mortality

During the follow-up time of 7.2 ± 2.3 years, 35 SSc patients (23%) died. No patient was lost to follow-up. The leading cause of death was

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