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GALECTIN-3 IN HEART TRANSPLANT REJECTION AND FIBROSIS

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Aim: to study plasma galectin-3 levels in heart recipients and to determine the potential significance of galectin-3 level in acute transplant rejection and fibrosis. **Methods.** The study included 107 heart transplant recipients, aged 16 to 70 (48 ± 13) years, of which 90 (84%) were men. Dilated cardiomyopathy was diagnosed in 57 patients prior to heart transplantation, end-stage ischemic heart disease in 50. Galectin-3 concentrations and placental growth factor (PlGF) were measured using enzyme-linked immunosorbent assay (ELISA); vascular endothelial growth factors (VEGF-D and VEGF-A), monocyte chemoattractant protein-1 (MCP-1), platelet-derived growth factors (PDGF-BB), and soluble CD40 ligand (sCD40L) were measured using multiplex technology xMAP. Acute graft rejection and myocardial fibrosis were verified through morphological examination of endomyocardial biopsy specimens. **Results.** Galectin-3 concentrations in patients with congestive heart failure (15.92 [11.80; 23.65] ng/ml) were significantly higher than in healthy individuals (11.08 [7.71; 14.47] ng/ml), p = 0.00. No correlation was found between galectin-3 levels and sex, age and pre-transplant diagnosis. A month after transplantation, plasma galectin-3 level was significantly higher than before transplantation; a year later, the levels decreased to pre-transplant levels (18.71 [13.14; 25.41] ng/ml). By the end of the first year after transplantation, the levels were significantly higher both in patients with 1-2 episodes and in the patients after 3 or more episodes of acute rejection, in contrast to recipients who were not diagnosed with rejection. By the end of the first year after heart transplantation in patients with fibrosis, plasma galectin-3 levels were significantly higher than in patients without fibrosis. By the end of the first year after heart transplantation, galectin-3 levels in the recipients were associated with the nature of myocardial fibrosis: in patients with diffuse focal fibrosis (22.52 [20.98; 26.08] ng/ml), plasma concentrations of galectin-3 were significantly higher than in patients without fibrosis (15.36 [11.95; 22.42] ng/ml, p = 0.01). **Conclusion.** Plasma levels of galectin-3 in heart recipients by the end of the first year after transplantation is associated with previous crises of acute graft rejection, irrespective of the number of rejection episodes. Elevated plasma levels of galectin-3 in heart recipients in the long term after transplantation is associated with myocardial fibrosis; galectin-3 levels are associated with the morphological characteristic of fibrosis in the transplanted heart (diffuse focal fibrosis).

Keywords: heart transplantation, biomarkers, galectin-3, myocardial fibrosis, rejection.

Despite all the recent improvements in immunosuppressive and adjuvant drug therapy and significant progress in the survival of patients with heart transplants, the recipients often develop an asymptomatic heart failure (HF) in the long-term. One of the key elements of the HF pathogenesis is fibrosis of the transplant myocardium resulting from the accumulation of the fibrillar collagen fragments. The HF development can also be caused by the rejection of a heart transplant, arterial hypertension, transplant vasculopathy, concomitant diseases, including metabolic syndrome, diabetes mellitus, impaired renal function, etc. [1–3].

Currently, a specific attention is paid to the identification of profibrogenic biological agents – biomarkers which can induce fibrosis on the one hand, and on the other, can act as indicators of the adverse events risk associated with its development. These biomarkers include the transforming growth factor β1, a marker of fibroblast activation, as well as the N-terminal region of the brain natriuretic peptide (NT-proBNP) synthesized in cardiomyocytes and fibroblasts [4–6]. The abovementioned markers are the best known and acknowledged in clinical practice. Galectin-3 is a recently described indicator of the chronic HF risk in patients, including cardiac re-

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cipients [7–9]. Galectin-3 is expressed by neutrophils, macrophages, eosinophils, osteoclasts, and myocardial fibroblasts. It is suggested that measuring Galectin-3 levels, along with other HF biomarkers and myocardial damage, may be of prognostic value for the long-term condition of the recipients after cardiac transplantation [10].

This paper studies the Galectin-3 plasma level in the heart transplant recipients and determines the potential significance of the Galectin-3 level in the acute rejection and transplantation fibrosis.

MATERIALS AND METHODS

107 patients participated in the study. From 2013 to 2016, they underwent heart transplantation (HT) at Shumakov National Medical Research Center of Transplantology and Artificial Organs of the Ministry of Health of Russia (NMRC TAO), they included 90 (84%) men; the average age of the recipients was 48 ± 13 (16 to 70) years. In 57 and 50 patients, dilated cardiomyopathy (DCM) and a coronary heart disease (CHD), respectively, caused a terminal heart failure which served as the basis for the transplantation. The maximum duration of the follow-up after HT reached 398 with a median of 347 [289; 364] days. The control group was represented by healthy adults (n = 10) with no difference in sex and age in comparison with the study group.

All the patients with HT indications went through the routine examination according to the protocol of patient management at NMRC TAO and the National Clinical Recommendations "Heart Transplantation and Mechanical Support for Blood Circulation". The routine examinations after HT included clinical assessment of the condition, full blood count and biochemical blood assay with determination of the tacrolimus concentration, daily blood pressure monitoring (for adjustment of antihypertensive therapy), echocardiographic examination, repeated myocardial biopsies, annual coronary angiography. All the recipients underwent three-component immunosuppressive therapy, which included a combination of calcineurin inhibitors (tacrolimus) and cytostatics (mycophenolate mophetyl or mycophenolic acid), as well as prednisolone orally depending on the period of time which passed from the date of surgery and frequency of rejection episodes and adjuvant drug therapy as clinically indicated [2, 3].

Acute cellular rejection of a heart transplant was diagnosed based on the results obtained from the histological, humoral – immunohistochemical test of endomyocardial biopsy samples. Endomyocardial biopsy (EMB) in cardiac recipients was conducted according to the protocol for routine clinical and laboratory examination or as indicated. The study of the biopsy sample aimed at determination of the fibrotic changes in the transplant and their nature (diffuse, focal, and diffuse focal fibrosis).

The venous plasma was used as the material for the study of biomarker concentration; a total of 233 samples were tested (1–3 samples from each patient, on average of 2.1 ± 0.6). Galectin-3 concentration was measured by enzyme immunoassay using a Human Galectin-3 Platinum ELISA reagent set (Bender MedSystems GmbH, Vienna, Austria). Placental growth factor (PlGF) was measured by enzyme immunoassay using the RandD SYSTEMS reagent sets, USA. Concentrations of vascular endothelial growth factor (VEGF-D and VEGF-A), monocyte chemoattractant protein-1 (MCP-1), platelet growth factor (PDGF-BB), and soluble ligand CD40 (sCD40L) were measured using xMAP technology with a multiplex panel based on Simplex ProcartaPlex™ reagent sets (Affymetrix, USA).

Data analysis and processing were made by the IBM SPSS STATISTICS 20 (IBM SPSS Inc., USA) scientific and engineering calculations software package. The data are given as the arithmetic mean and standard deviation (M ± SD) for parametric methods and as the median and interquartile range for nonparametric methods. Statistical processing of the obtained data engaged the methods of nonparametric statistics: when comparing dependent samples, the Wilcoxon paired test was calculated, and the Mann–Whitney U-test was used to compare the independent variables. For all the criteria, the critical significance level was assumed to be 5%, i. e. the null hypothesis was rejected at $p < 0.05$.

RESULTS AND DISCUSSION

In patients with terminal-stage HF, the range of Galectin-3 concentrations in plasma varied widely and corresponded to a nonparametric distribution. The median of Galectin-3 concentrations in patients with HF were higher than in healthy subjects (11.08 ng/ml, interquartile range – [7.71; 14.47] ng/ml) and reached 15.92 [11.80; 23.65] ng/ml, p = 0.00 (Fig. 1).

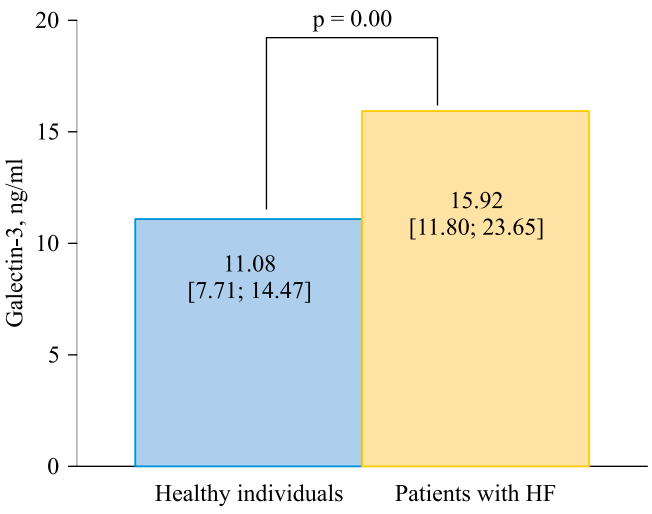
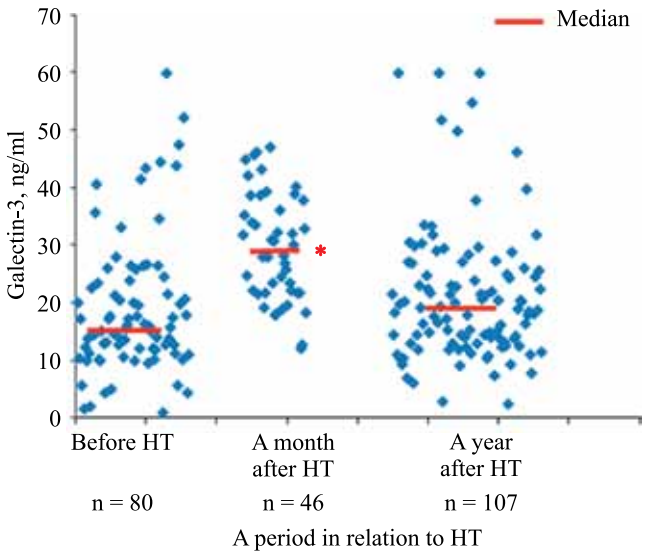


Fig. 1. Comparative analysis of galectin-3 plasma concentration of healthy individuals and patients with terminal heart failure

In men, the Galectin-3 level was 15.76 [11.80; 23.65] ng/ml and was not significantly different from that in women – 18.46 [12.46; 21.56] ng/ml, (p = 0.69). Galectin-3 levels were independent of the patient age. No differences in Galectin-3 concentrations were observed in patients with DCM 12.21 [12.12; 23.65] ng/ml and CHD 15.81 [10.92; 23.48] ng/ml (p = 0.77).

One month after HT, the Galectin-3 level in the recipients' plasma amounted to 29.21 [21.97; 37.44] ng/ml proved to be significantly higher than in patients before HT. The potential reason of higher level of Galectin-3 in the first month after HT is a complex of factors associated with surgery, early postoperative period, including systemic inflammatory response to surgery; adaptation of the recipient's organism to the transplanted organ and immunosuppressive therapy, etc.

By the end of the first year after HT, the level of Galectin-3 in patients had decreased to the pretransplantational level and amounted to 18.71 [13.14; 25.41] ng/ml (Fig. 2).



* – p < 0,05, compared with level before transplantation.

Fig. 2. The level of galectin-3 in patients before heart transplantation and recipients in early and late period after heart transplantation

Table

Correlation between the Galectin-3 plasma level in heart recipients and the level of biomarkers, potentially relevant for the diagnosis of post-transplant complications

Marker	Correlation rate, r	Confidence, p-value
VEGF-A	–0.004	0.98
VEGF-D	**–0.511**	**0.00**
PlGF	**0.293**	**0.04**
PDGF-BB	–0.208	0.15
MCP-1	**–0.285**	**0.05**
sCD40L	–0.162	0.27

The recipients' Galectin-3 plasma concentration a year after HT correlated with levels of other biomarkers potentially relevant for the diagnosis of post-transplant complications. A positive correlation of Galectin-3 concentration with the PlGF level was revealed (r = 0.293, p = 0.04) while a negative correlation was observed for VEGF-D (r = –0.511, p = 0.00) and MCP-1 (r = –0.285, p = 0.05) (Table).

The results of the analysis of the correlation between Galectin-3 plasma level in recipients and the number of episodes of acute transplant rejection are as follows.

By the end of the first month after heart transplantation, no significant difference in the median of Galectin-3 concentrations was observed in patients who suffered an acute cellular (n = 27) and humoral (n = 1) rejection and had no rejection episodes (n = 18) during the early post-transplantation period.

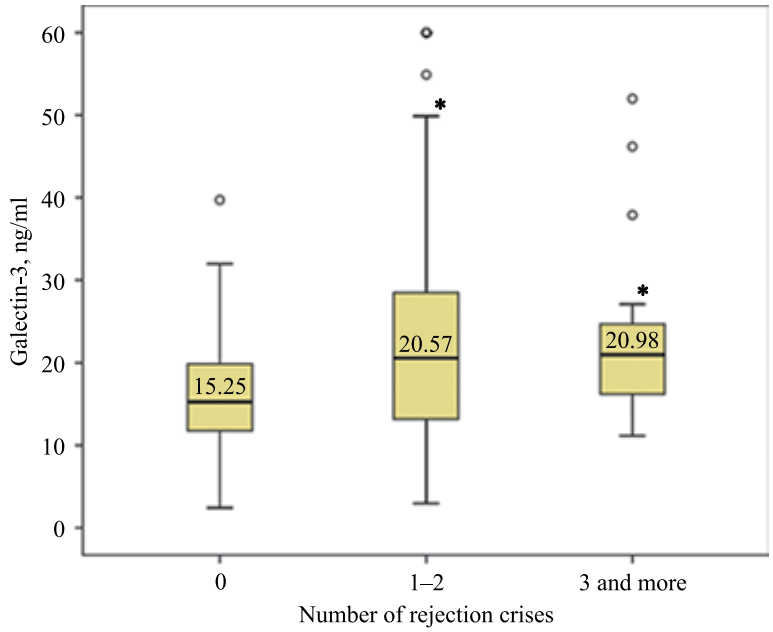
By the end of the first year after heart transplantation, 75 out of 107 participated patients had an acute rejection crises: 57 patients suffered 1–2 episodes (acute cellular rejection, n = 54 and humoral rejection, n = 3) and 18 patients had 3 and more episodes of rejection (acute cellular rejection, n = 14 and humoral rejection, n = 4). The level of Galectin-3 was significantly higher in patients with rejection crises, in contrast to the recipients not diagnosed with such crises. The Galectin-3 level did not depend on the number of crises and was higher both in patients who experienced 1–2 episodes and in those who had 3 or more rejections (Fig. 3).

Histological examination of endomyocardial biopsy samples by a qualitative imaging method was used to assess the presence, severity and nature of fibrosis as a manifestation of pathological changes in the myocardium.

A month after the HT, 32 samples with pathological changes in the myocardium, indicating fibrosis of varying severity, and 14 samples without such changes were found among the tested endomyocardial samples from 46 recipients. By the end of the first month, no reliable differences were found in the median of Galectin-3 plasma concentrations in recipients with revealed fibrosis and without it, although a higher Galectin-3 level tended to be observed in patients with fibrosis than without it (30.55 and 26.39 ng/ml, respectively, p = 0.86).

By the end of the first year after HT, fibrotic myocardial changes were revealed in 64 of the 107 studied biopsy samples. The median of Galectin-3 concentrations in recipients with the transplanted heart myocardial fibrosis reached 20.60 [14.52; 26.29] ng/ml, in recipients without fibrosis – 15.36 [11.95; 22.42] ng/ml; (p = 0.05) (Fig. 4).

All the biopsy samples with sclerotic myocardial changes underwent a qualitative assessment of the fibrosis severity. Fig. 5, a shows an example of a histological specimen of a heart with no fibrotic changes. Diffuse fibrosis, which develops in the interstitial or perivascular space and is not associated with a significant loss of functioning cells, was found in 16 recipients (Fig. 5, b).



* – p < 0,05, compared with recipients without rejection.

Fig. 3. The levels of galectin-3 concentration in recipients one year after heart transplantation, depending on the number of acute rejection episodes

Focal fibrosis with a replacement of the dead cardiomyocytes with connective tissue was found in 38 recipients (Fig. 5, c). The most severe form of fibrosis, diffuse focal fibrosis, was found in 10 recipients.

The results of the study showed that the Galectin-3 plasma level in recipients at the end of the first year after HT is associated with the nature of pathological myocardium changes. In patients with diffuse focal fibrosis, Galectin-3 level was significantly higher compared with patients without fibrosis (22.52 [20.98; 26.08] ng/ml, p = 0.01). No significant difference was found in patients with diffuse or focal fibrosis compared with no fibrosis group (18.69 [14.31; 26.14] ng/ml and 19.13 [14.36; 25.81] ng/ml, respectively. p > 0.05) (Fig. 6).

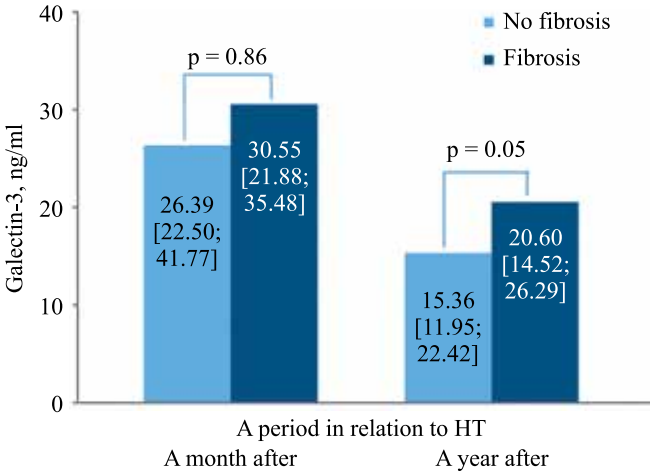


Fig. 4. Comparative analysis the concentration of galectin-3 in the cardiac recipients the early and long-term periods after transplantation with and without morphological signs of myocardial fibrosis

CONCLUSION

The results of this study showed that the Galectin-3 concentration in patients suffering from the heart failure

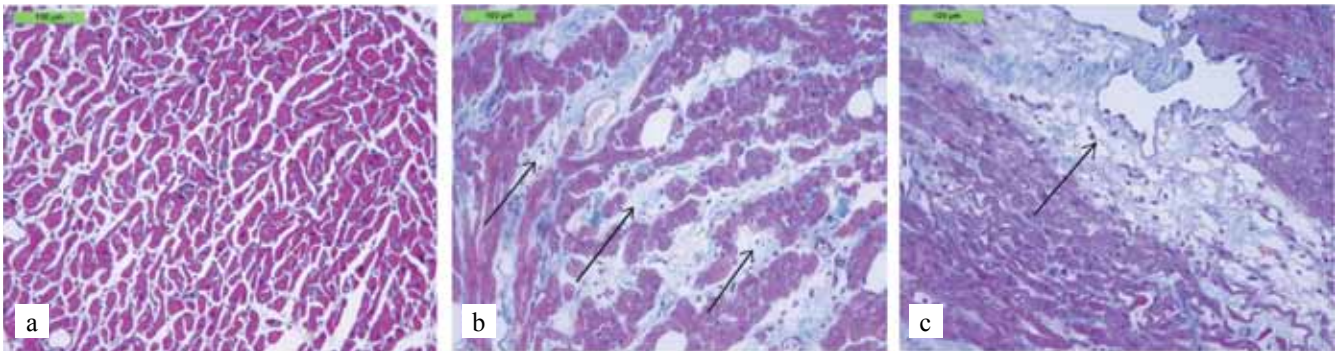
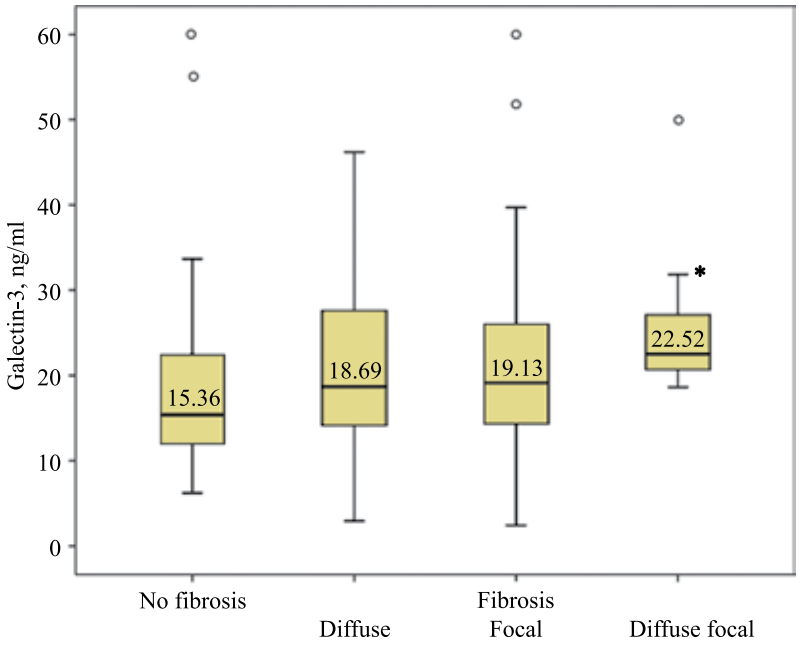


Fig. 5. Histological examination of endomyocardial biopsy specimens: a – focal protein dystrophy of cardiomyocytes, focal moderate edema of interstitium, no fibrosis; b – diffuse moderate protein dystrophy of cardiomyocytes, diffuse fibrosis; c – protein dystrophy of cardiomyocytes, focal fibrosis. Coloring according to Masson. ×200



* – p = 0,01, compared with patients without myocardial fibrosis.

Fig. 6. Comparative analysis median concentration of galectin-3 by the end of first year after transplantation in cardiac recipients with different types of fibrosis and without it

in its terminal stage (and potential heart recipients) is higher than in healthy subjects. In the early post-transplant period, Galectin-3 concentration exceeded its pretransplantational value. This fact may be associated with the factors of the early postoperative period; a year later, the average plasma Galectin-3 level in the recipients did not differ from the pretransplantational level.

By the end of the first year after transplantation, Galectin-3 level was significantly higher in the recipients who suffered acute transplant rejection crises, regardless of their number: the patients who had 1 to 2 crises and those who suffered 3 or more rejections had no difference in Galectin-3 plasma concentrations.

In the recipients with morphological signs of myocardial fibrosis, the Galectin-3 level was significantly higher compared with no fibrosis group. It was associated with the nature of pathological changes in the myocardium (with diffuse focal fibrosis).

The authors declare no conflict of interest.

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