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DIAGNOSTIC VALUE OF GALECTIN-3 IN HEART TRANSPLANT RECIPIENTS WITH MYOCARDIAL COMPLICATIONS

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Objective: to determine the diagnostic value of galectin-3 in transplant recipients with myocardial fibrosis and acute heart transplant rejection, verified by endomyocardial biopsy. **Materials and methods.** The study included 124 patients with end-stage heart failure. Their ages ranged from 16 to 71 (average 48 ± 12) years, of which 106 (85%) were men and 18 (15%) were women. From 2013 to 2016, these patients underwent a heart transplant procedure at the Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation. Analysis of endomyocardial biopsy specimens was used to verify acute cellular, humoral rejection and myocardial fibrosis of the heart transplant. Severity and nature of fibrosis was evaluated using a qualitative imaging technique. Galectin-3 concentration was measured by enzyme immunoassay using Human Galectin-3 Platinum ELISA reagent kits (Bender MedSystems GmbH, Vienna, Austria). **Results.** In the long-term post-transplantation period, in comparison with the early post-transplantation period, the number of verified graft myocardial fibrosis increased by 88% in recipients who had acute rejection crises and by 37% in recipients who had no rejection crises. Graft myocardial fibrosis was detected more often in recipients who had antibody-mediated rejection than in those who had acute cell rejection (92% vs 75% of cases, respectively). Plasma galectin-3 levels in recipients with graft myocardial fibrosis was higher than in recipients without it ($p = 0.05$ 1 year and $p = 0.01$ 1–5 years after heart transplantation). In recipients who had acute rejection crises, the risk of developing graft myocardial fibrosis was 1.64 (RR = 1.64 ± 0.1 [95% CI 1.1–2.2]). **Conclusion.** Galectin-3 is a biomarker for myocardial fibrosis in acute heart transplant rejection.

Keywords: heart transplantation, galectin-3, myocardial fibrosis, acute rejection, diagnostic value.

INTRODUCTION

The current achievements in heart transplantation (HT) have provided higher survival and improved quality of life for recipients. In the long term after transplantation, heart recipients have an increased risk of developing subclinical chronic heart failure, resulting from a combination of various pathological factors leading to the formation of transplant myocardial fibrosis, such as arterial hypertension, acute transplant rejection, transplant vasculopathy, and other diseases [1].

Endomyocardial biopsy (EMB) is an objective method for verification of myocardial pathology. After transplantation, EMB is performed within the time required by the treatment protocol or as indicated; however, there are some limitations and risks inherent in all invasive diagnostic methods. Moreover, when examining a biopsy sample, a myocardial fragment is evaluated which may not reflect the state of other areas that did not fall into the test material. The development of minimally invasive methods for identifying complications in the post-transplant period is performed, with the aim of improving preclinical diagnosis, considering, among others, the need to reduce the number of repeated invasive diagnostic interventions, partially replacing them with functional and/or laboratory tests which can not only detect the presence of transplant myocardial fibrosis, but also control the effectiveness of the recipient treatment.

Particular attention is paid to the identification of profibrogenic biological agents that can be indicators of the risk of negative cardiovascular events associated with the development of fibrosis [2, 3]. The relatively recently described biomarkers for the development of heart failure and myocardial fibrosis include galectin-3 which belongs to the lectin family and plays an important role in the regulation of myofibroblast proliferation, immune response, inflammation and remodeling of heart vessels [4, 5]. At the site of injury, galectin-3 is secreted into the extracellular space and promotes the develop-

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ment of fibrosis through the activation of fibroblasts [6]. In the role of heart recipients, the role of galectin-3 has been less studied, but it has been found that its level in blood plasma is higher in patients with graft myocardial fibrosis [7]. The aim of this study was to determine the diagnostic efficacy of galectin-3 in recipients with myocardial fibrosis and acute graft rejection, verified by endomyocardial biopsy.

MATERIALS AND METHODS

The study included 124 patients with heart failure of the III–IV functional class according to the classification of the New York Heart Association (NYHA) aged 16 to 71 (average 48 ± 12), 106 (85%) men and 18 (15%) women. In 2013 to 2016, at the Shumakov National Medical Research Center of Transplantology and Artificial Organs (Moscow, Russia), the patients have got heart transplants. In 67 recipients (51 men and 16 women, aged 16 to 71; 41 ± 12), heart failure was caused by dilatation cardiomyopathy, and 57 (55 men and 2 women, aged 37 to 70; 57 ± 8 years old) had coronary artery disease (CAD).

All patients indicated for HT underwent a routine examination in compliance with the protocol of patient management at the Shumakov National Medical Research Center of Transplantology and Artificial Organs and National Clinical Recommendations [8]. Routine examination of recipients included clinical examination, thermometry, virologic and bacteriological analyses, CBC and blood biochemistry in dynamics with determination of tacrolimus concentration, daily monitoring of blood pressure, echocardiography, myocardial biopsy, and annual coronary angiographic examination.

Acute cellular and humoral rejection, as well as transplant myocardial fibrosis, were verified on the basis of a study of endomyocardial biopsy samples. For histological examination, pieces of endomyocardium were fixed in 10% formalin, then washed with water, dehydrated, and embedded in paraffin. 3–4 microns thick slices were prepared on a microtome. To verify myocardial fibrosis of the transplant, slices were Masson Trichrome stained which made it possible to clearly distinguish between connective tissue, which, depending on its maturity, is stained in various shades of blue and differs from other myocardial tissues.

Fig. 1 shows examples of histological preparations of transplanted heart biopsy specimens where fibrotic changes of various types are detected: focal, diffuse, and diffuse focal fibrosis.

To diagnose the acute cellular rejection (ACR), the slices were hematoxylin and eosin stained; to diagnose the antibody-mediated rejection (AMR), immunohistochemical tests were used. The degree of acute cellular and humoral transplant rejection was evaluated according to the recommended classifications adopted by the International Society for Heart and Lung Transplantation (ISHLT-2004 and ISHLT-2013).

Venous blood plasma served as a material for studying the concentration of galectin-3. The concentration of galectin-3 was measured by enzyme immunoassay with the Human Galectin-3 Platinum ELISA reagent kits (Bender MedSystems GmbH, Vienna, Austria).

Sensitivity and specificity, the selection of the optimal cutoff threshold and of the best diagnostic strength test were determined by ROC analysis. To assess the diagnostic significance of galectin-3, a relative risk indicator was used.

The analysis of the obtained data was performed by the standard statistical processing methods with Microsoft Office Excel and the IBM SPSS STATISTICS 20 software package for scientific and technical calculations (IBM SPSS Inc., USA).

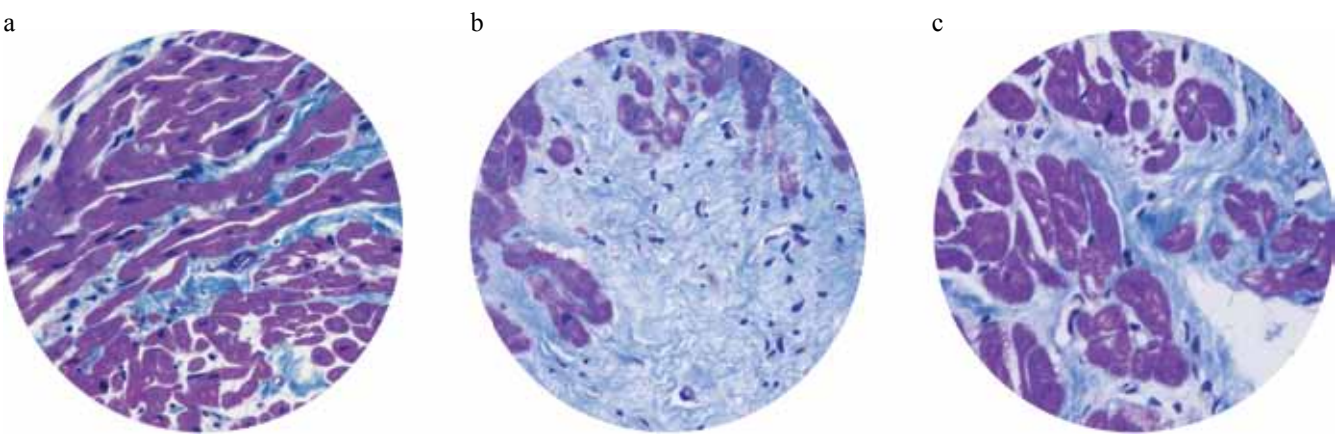

Fig. 1. Histological preparations of endomyocardial biopsy specimens. Coloring according to Masson ×400 (connective tissue is colored blue, cardiomyocytes are pink): a – diffuse growth of loose fibrous connective tissue with single cells of the fibroblastic line, focal protein granular dystrophy of cardiomyocytes; b – focal growth of unformed connective tissue with single cells of the connective tissue row, moderate protein dystrophy of cardiomyocytes; c – diffuse focal growth of loose fibrous connective tissue, in which proliferation of connective tissue cells is noted, focal protein dystrophy of cardiomyocytes

RESULTS AND DISCUSSION

The histological signs of transplant myocardial fibrosis at different times after HT were detected in 124 recipients selected by random sampling from a total of 432 recipients who underwent HT in 2013–2016 at the Shumakov National Medical Research Center of Transplantology and Artificial Organs. 583 endomyocardial biopsies (from one patient: from 3 to 20; on average 5 ± 2 EMB) obtained in the following periods after transplantation: early period – the first month after HT (30 ± 14 days), a year later (334 ± 69 days) and after 1–5 years (963 ± 273 days) were studied.

By the end of the first month after HT, 58 (46%) of the heart recipients included in the study showed transplant myocardial fibrosis; by EMB, 66 (54%) recipients showed no histological signs of fibrosis. One year after HT, 74 (60%) recipients, and 1–5 years later, 95 (77%) recipients developed verified transplant myocardial fibrosis (Fig. 2).

The presented data reflect the rate of detecting fibrotic changes in the graft myocardium in the randomly selected recipients of the studied group and cannot be extrapolated to the entire population of heart recipients operated at the Shumakov National Medical Research Center of Transplantology and Artificial Organs in 2013–2016. At the same time, the results allow us to state, firstly, a fairly frequent, in almost half of cases, detection of transplant myocardial fibrosis even in the early period after HT; secondly, we can note the significant increase in the proportion of cases of transplant myocardial fibrosis in recipients with the time after HT: in the present study, by 67% after 1–5 years compared with the early period.

Obviously, in the early post-transplant period, the cause of myocardial fibrosis may be the presence of fibrotic changes in the myocardium of the donor heart. It should be noted that the age of the heart donors for the recipients included in this study was 42 ± 11 (18 to 64) years. Studies of the donor heart to detect fibrotic changes have not been performed; however, the implementation of such an analysis will be appropriate to study the factors affecting the development of transplant myocardial fibrosis and the long-term prognosis of heart recipients.

The analysis of the rate of detection of various types of fibrosis in the early and long-term periods after HT showed that by the end of the 1st month after transplantation of 58 recipients with transplant myocardial fibrosis, diffuse fibrosis was verified in 13 (22%), focal fibrosis in 40 (69%) and 5 (9%) – diffuse focal fibrosis. After 1–5 years after HT, diffuse fibrosis was verified in 20 (21%) of 95 recipients with myocardial fibrosis, focal in 55 (58%) and diffuse-focal fibrosis in 20 (21%). Diffuse fibrosis develops in the interstitial or perivascular space, it is accompanied by excessive deposition of type I collagen in the myocardium due to the predominance of its synthesis over decay [9]. With the development of focal fibrosis, dead cardiomyocytes are replaced by connective tissue and are accompanied by excessive deposition of type III collagen [10]. According to the results of this study, there is an increase in the proportion of the most severe, diffuse focal forms of fibrosis in the study group of recipients: from 9% in the early post-transplant period to 21% in the long term.

Earlier, it has been previously found that the level of galectin-3 in blood plasma is higher in recipients with graft myocardial fibrosis; higher values were found in diffuse focal fibrosis [7]. To determine the diagnostic significance of galectin-3, the ROC curve analysis was used as a marker of transplant myocardial fibrosis (Fig. 3).

Calculations showed that the area under the ROC curve of galectin-3 1–5 years after transplantation in recipients with transplant myocardial fibrosis was 0.765 ± 0.060 [0.64–0.88], p = 0.00.

The threshold levels of galectin-3 significant for the diagnosis of transplant myocardial fibrosis 1–5 years after heart transplantation, were determined at a point by the optimal combination of sensitivity and specificity. In the diagram of the dependences of sensitivity and specificity on the concentration of galectin-3 in blood plasma, the intersection point of the curves reflects the threshold level (Fig. 4).

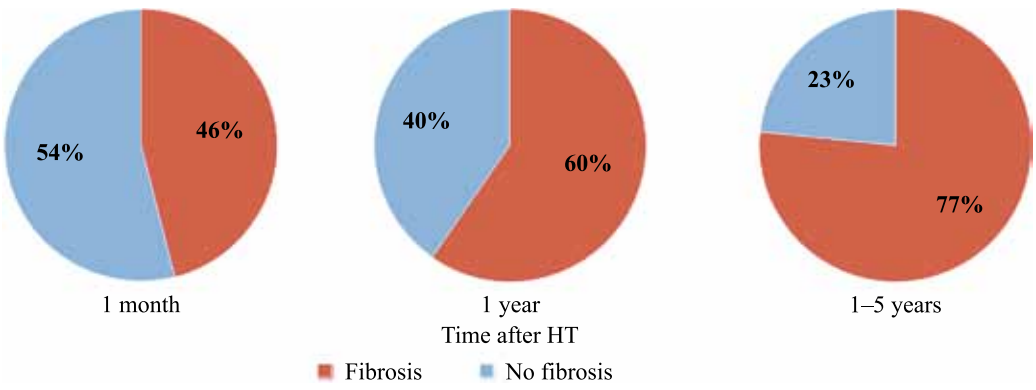

Fig. 2. Percentage of verified myocardial fibrosis in the recipients at different times after HT

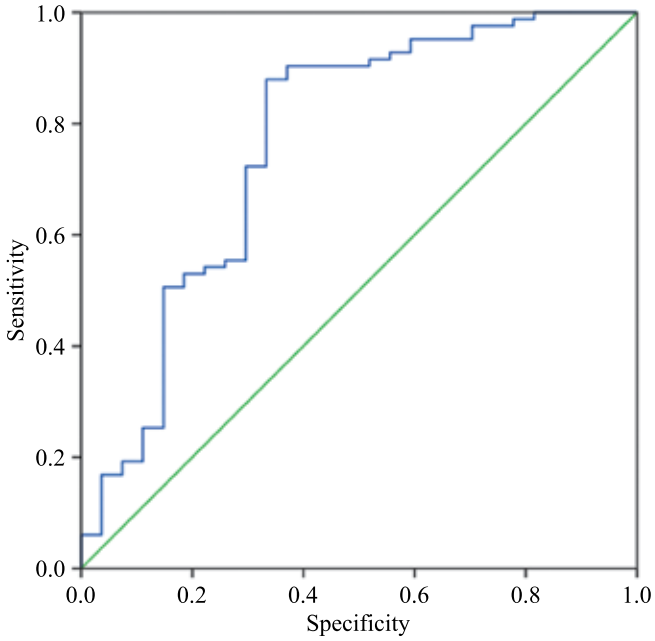

Fig. 3. Galectin-3 ROC curve 1–5 years after HT in recipients with transplant myocardial fibrosis

The threshold value of galectin-3 significant for the diagnosis of transplant myocardial fibrosis in the long term after HT, was 16.9 ng/ml. At a galectin-3 level exceeding the found threshold value, the probability of transplant myocardial fibrosis risk in heart recipients is 1.6 times higher (RR = 1.6 ± 0.1 [95% CI 1.2–2.0]), than in recipients with a galectin-3 level below this threshold value (sensitivity – 71%, specificity – 70%).

Among the main factors limiting the survival of heart recipients in the early postoperative period and during the first year of life after HT is acute rejection of the transplanted heart. The reaction of rejection of a heart transplant is a manifestation of the protective reaction of the recipient's organism against foreign cells of the donor organ, includes mechanisms of an innate, cellular and antibody-mediated (humoral) immune response, as a result of which episodes of acute transplant rejection are a factor stimulating the development of fibrotic changes in the transplanted heart.

Among the 124 patients included in this study, 75 (60%) recipients suffered episodes of acute rejection of the transplanted heart in the early post-transplant period. A year after transplantation, already 89 (72%), and after 1–5 years – 92 (74%) of heart recipients suffered acute rejection episodes.

Of the patients who suffered acute transplant rejection episodes, myocardial fibrosis in the early stages after HT was detected in 31 (41%) of 75 recipients; a year later, in 51 (57%) of 89 recipients; after 1–5 years, it was verified in 71 (77%) of 92 recipients.

In patients without acute rejection episodes, graft myocardial fibrosis was detected in 27 (55%) of 49 heart recipients in the early stages; a year later, myocardial fibrosis was verified in 23 (65%) of 35 recipients; after 1–5 years – in 24 (75%) of 32 recipients.

Although in the long term after transplantation, the proportion of recipients with verified myocardial fibrosis practically did not differ in the groups who underwent and did not undergo acute rejection (77% and 75%, respectively), the analysis showed that in recipients of the heart who underwent acute rejection episodes, the proportion of recipients with myocardial fibrosis after 1–5 years compared with the early period after transplantation increased by 88%, and in recipients who did not suffer acute rejection episodes – by 37% (Fig. 5).

The relationship between the episodes of antibody-mediated rejection and the development of transplant myocardial fibrosis is more pronounced: in recipients of the heart who underwent humoral rejection, transplant myocardial fibrosis is detected in long-term periods in

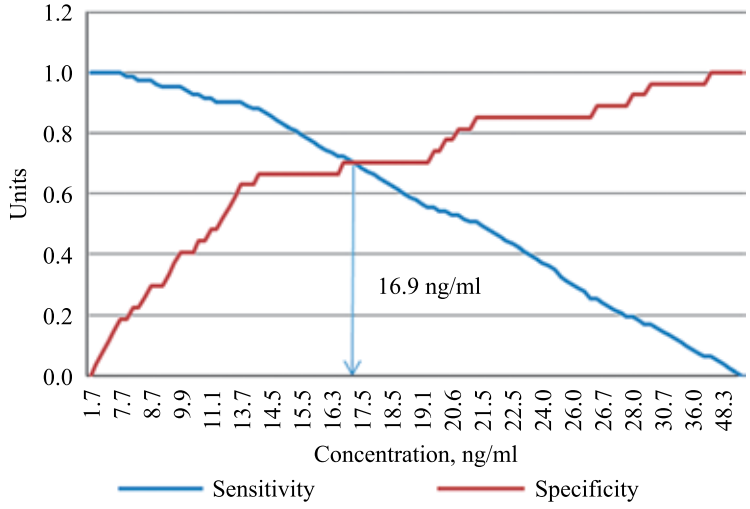

Fig. 4. The diagnostic significant threshold value of the galectin-3 1–5 years after HTx for transplant myocardial fibrosis

92% of cases, and in recipients who have experienced acute cell rejection episodes – in 75% of cases (Fig. 6).

The detected differences seem to be associated with an additional negative effect of immune factors acting upon humoral rejection of the graft myocardium.

The results of the present study confirm the idea of the effect of episodes of acute cellular and antibody-mediated rejection on the development of fibrotic changes in a transplanted heart. Obviously, the formation of the latter is promoted by edema developing during acute rejection, macrophage and lymphocyte infiltration, production of activated inflammatory cells and fibroblasts of pro-inflammatory and profibrogenic mediators, etc. [11, 12].

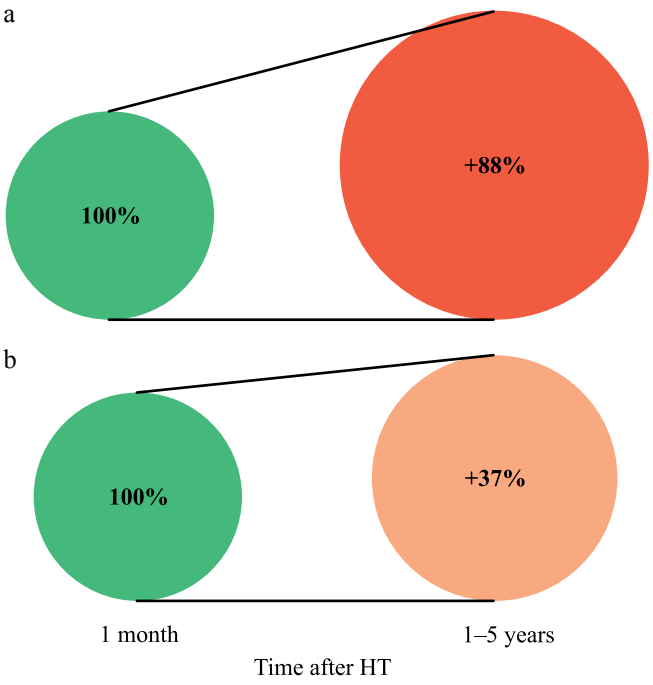

Fig. 5. An increase in the number of verified cases of graft myocardial fibrosis in recipients with (a) and without (b) episodes of acute rejection

A comparative analysis of the diagnostic efficacy of galectin-3 in myocardial fibrosis in recipients who underwent and did not undergo acute transplant rejection showed the following. In the long term after HT, the concentration of galectin-3 in recipients who underwent and did not undergo acute transplant rejection was 20.57 [13.92; 27.24] and 15.25 [12.06; 19.47] ng/ml, respectively, p = 0.00. In patients with transplanted heart myocardial fibrosis and without fibrosis, according to EMB, the level of galectin-3 was 20.60 [14.52; 26.29] and 15.36 [11.95; 22.42] ng/ml, respectively, p = 0.05 [7].

At galectin-3 concentration exceeding the calculated threshold value (16.9 ng/ml), the relative risk of myocardial fibrosis in the long term after transplantation in recipients who underwent acute rejection episodes was RR = 1.64 ± 0.1 [95% CI 1.1–2.2] (sensitivity – 71%, specificity – 75%).

In recipients with galectin-3 level above 16.9 ng/ml but not suffering acute rejection, the relative risk of developing myocardial fibrosis was RR = 1.38 ± 0.2 [95% CI 0.8–2.3] (sensitivity – 71%, specificity – 57%) and was not statistically significant (the boundaries of the confidence interval included one).

Thus, in the long term after HT, galectin-3 concentration in the blood plasma has diagnostic value in rela-

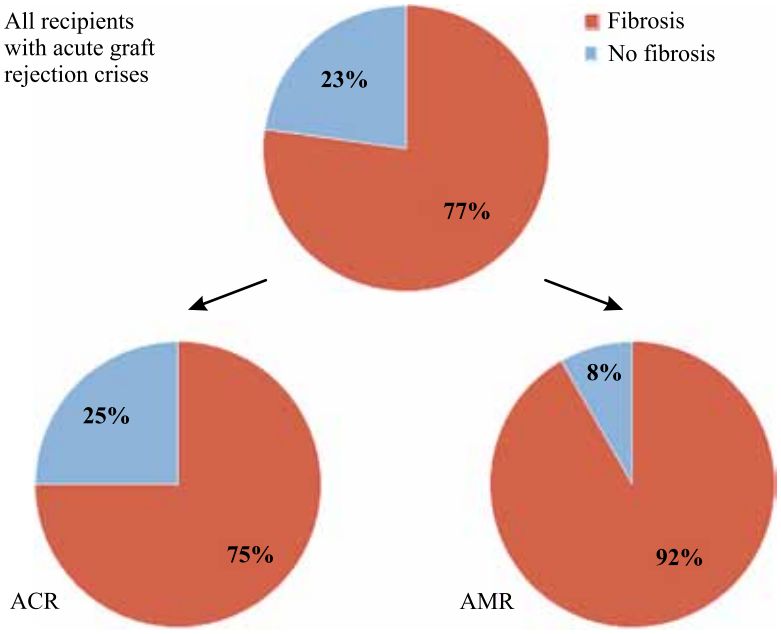

Fig. 6. The frequency of detection of myocardial fibrosis (%) after 1–5 years after HT in recipients with acute cellular (ACR) and humoral rejections (AMR) graft episodes

tion to transplant myocardial fibrosis in recipients who underwent acute rejection crises. At the same time, in recipients who did not undergo rejection crises, the test for galectin-3 to detect transplant myocardial fibrosis is insignificant. Most likely, the latter is associated with a lesser activity of the processes of fibrogenesis that occur in the body of recipients who have not undergone acute rejection, which is also indicated by differences in the increase in the number of verified cases of graft myocardial fibrosis in recipients who underwent (by 88%) and who did not (37%) crises of acute rejection, for a period of 1 month to 1–5 years after HT. In recipients of the heart who have suffered crises of acute transplant rejection, at galectin-3 ≥16.9 ng/ml, the risk of developing myocardial fibrosis is 1.64 times higher than in recipients with a galectin-3 level below the threshold.

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The authors declare no conflict of interest.

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