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DIAGNOSTIC VALUE OF MICRORNA-27 AND -339 IN HEART TRANSPLANT RECIPIENTS WITH MYOCARDIAL FIBROSIS

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Myocardial fibrosis plays a key role in the pathogenesis of heart failure. A family of small non-coding signaling molecules, microRNAs (miRNAs), has been identified as promising profibrogenic biomarkers capable of signaling a possible risk of adverse events after heart transplantation. **Objective:** to identify and evaluate the diagnostic significance of miRNAs, as well as comprehensive miRNA-based tests in heart recipients with graft myocardial fibrosis. **Materials and Methods.** The study included 83 heart recipients aged 16 to 64 (48.4 ± 13.1) years. The expression levels of five microRNAs (miR-27, -101, -142, -339, -424) in venous blood plasma were measured by quantitative real-time polymerase chain reaction; galectin-3 serum levels were determined by enzyme immunoassay. **Results.** Morphological signs of graft myocardial fibrosis were verified in 48 recipients. The miR-27 and miR-339 expression levels were significantly higher in heart recipients with myocardial fibrosis than in those without myocardial fibrosis ($p = 0.018$ and $p = 0.043$, respectively). Diagnostically significant threshold levels of miR-27 and miR-339 for detection of myocardial fibrosis in heart transplant recipients were determined (–4.33 and –5.24 units, respectively). The relative risk of detecting graft myocardial fibrosis in recipients with miR-27 expression value above the threshold level was RR = 1.5 ± 0.157 [95% CI 1.104-2.039], $p = 0.009$; for miR-339, RR = 1.31 ± 0.130 [95% CI 1.018-1.692], $p = 0.036$. When miR-27 expression levels and galectin-3 serum levels simultaneously exceeded their estimated thresholds, the risk of transplanted heart myocardial fibrosis increased to RR = 2.7 ± 0.456 [95% CI 1.090-6.524], $p = 0.032$; when miR-339 and galectin-3 simultaneously exceeded threshold values, the risk was RR = 2.0 ± 0.316 [95% CI 1.076-3.717], $p = 0.028$). **Conclusion.** The miR-27 and miR-339 expression levels are associated with the presence of fibrotic changes in the graft myocardium. The combination of molecular-genetic and proteomic biomarkers in one test improves the diagnostic characteristics of these expressions with respect to post-transplant complications in heart recipients.

Keywords: heart transplantation, myocardial fibrosis, microRNA-27, microRNA-339, galectin-3.

INTRODUCTION

Despite significant advances in heart transplantation (HTx), post-HTx recipients are at risk of developing subclinical chronic heart failure (CHF) due to graft fibrosis caused by accumulation of fibrillary collagen in the myocardium. Examination of endomyocardial biopsy specimens can detect graft pathology, but is severely limited by the invasiveness of this intervention.

In recent years, there has been an active development of minimally invasive methods for diagnosing post-transplant complications that will detect the presence of acute rejection, as well as other forms of cardiac graft pathology [1, 2].

Promising candidates for the role of such biomarkers are microRNAs – a family of small endogenous non-coding single-stranded RNAs acting as post-transcriptional gene regulators that play a key role in many biological processes [3].

To date, over 2000 microRNAs have been identified and their involvement in the functions of healthy and damaged cells has been confirmed. MicroRNAs are mostly tissue-specific and regulate expression of over 30% of genes. The diagnostic potential of evaluation of expression level of some microRNAs in blood samples of patients in relation to development and course of CHF has been shown [4]. Recent studies have shown that measuring the expression levels of certain types of microRNAs in solid organ recipients can be used to improve diagnosis of post-transplant complications, including fibrosis of heart, kidney, liver, and lung grafts [5, 6].

Our objective in this work is to identify and assess the diagnostic value of microRNAs, as well as compre-

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hensive tests based on them in heart recipients with graft myocardial fibrosis.

MATERIALS AND METHODS

The study included 83 randomly selected patients aged between 16 and 64 years (48.4 ± 13.1), who underwent HTx between 2013 and 2018 at Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, of whom 64 (77.1%) were male.

According to the protocol of patient management at Shumakov Center and the National Clinical Guidelines of the Russian Transplant Society, all recipients underwent routine examinations after HTx, which included clinical evaluation of their condition, general and biochemical blood tests with determination of tacrolimus levels, and repeated myocardial biopsies.

The expression of five microRNAs (miR-27, -101, -142, -339, -424), presumably playing a role in the development of cardiac graft pathology, was examined in venous blood plasma (1 to 3 samples from each recipient, mean 1.2). Blood samples were collected in disposable tubes with anticoagulant, centrifuged and the resulting plasma was frozen at –20 °C. Total RNA was isolated from 100 μL of blood plasma using Serum Plasma kits (Qiagen, USA) with preliminary addition of 1.6×10^8 copies of synthetic cel-miR-39 microRNA (Qiagen) after plasma incubation with Qiazol phenolic mixture. Cel-miR-39 was used as an internal control for RNA isolation efficiency, complementary DNA (cDNA) synthesis and real-time polymerase chain reaction (PCR). The expression intensity was calculated by the $2^{-\Delta\Delta CT}$ method [7] and expressed in relative units equivalent to $\log_2(2^{-\Delta\Delta Ct})$, where ΔCt is the working values of the change in the product cycle relative to the internal control of cel-miR-39 microRNA expression.

Galectin-3 levels were measured by enzyme-linked immunosorbent assay using the Human Galectin-3 Platinum ELISA reagent kits (Bender MedSystems GmbH, Austria) according to instructions accompanying them.

Signs of fibrotic changes in the graft myocardium were determined by histological examination of biopsy material. Endomyocardial biopsy (EMB) in recipients was performed during routine examination or as indicated in accordance with the protocol.

The magnitude of expression was assessed using Spearman's correlation and Mann–Whitney U test to compare independent variables. Differences in the compared groups were considered significant at $p < 0.05$. Sensitivity and specificity were determined by ROC analysis. A log-rank comparison of Kaplan–Meier survival curves for non-adverse events was performed in the recipients. The relative risk ratio (RR) was used to assess the diagnostic significance. The Youden index was calculated to determine the threshold of microRNA expression [8]. Sensitivity, specificity as well as the positive predictive value (PPV) and negative predictive value (NPV) of the tests were evaluated. Data were statistically processed using Statistica v.13.0, StatSoft Inc. (USA).

RESULTS

A series of studies on endomyocardial biopsy specimens obtained from 48 heart recipients revealed graft myocardial fibrosis.

Table 1 presents a comparative analysis of the expression levels of the investigated microRNAs in heart recipients with and without myocardial fibrosis.

When five microRNAs were examined, the expression levels of miR-27 and miR-339 were higher in patients with myocardial fibrosis than in recipients without fibrosis ($p = 0.018$ and $p = 0.043$ respectively).

The diagnostic significance of miR-27 and -339 was assessed by calculating the area under their ROC curves (AUC). Fig. 1 shows the ROC curves of miR-27 and -339 expression in heart recipients with graft myocardial fibrosis.

The area under the ROC curve for miR-27 was 0.69 ± 0.072 [95% CI 0.545–0.828] and significantly differed from 0.5 ($p = 0.010$); for miR-339, it also significantly differed from 0.5 – 0.67 ± 0.072 [95% CI 0.528–0.812] ($p = 0.019$).

The optimal combination of sensitivity and specificity values, corresponding to the highest Youden Index, determined the diagnostically significant threshold of miR-27 and miR-339 for detection of graft myocardial fibrosis (–4.33 and –5.24 relative units, respectively).

The log-rank method was used to assess the survival rate without adverse events in recipients with myocardial fibrosis and miR-27 and miR-339 expression levels above and below the calculated thresholds. Taken as

Table 1

MicroRNA expression levels in heart recipients with and without myocardial fibrosis

MicroRNA	Recipients with fibrosis	Recipients without fibrosis	p
miR-27	–5.414 [–6.430; –4.330]	–3.742 [–5.738; –1.576]	0.018
miR-101	–7.629 [–8.732; –5.913]	–5.844 [–7.452; –4.467]	0.105
miR-142	–6.925 [–8.297; –5.863]	–6.226 [–8.036; –5.152]	0.409
miR-339	–9.907 [–11.603; –7.784]	–7.925 [–10.132; –3.543]	0.043
miR-424	–6.532 [–7.779; –5.288]	–7.006 [–7.883; –5.617]	0.579

adverse events were death, heart retransplantation or balloon angioplasty.

Recipients with miR-27 expression levels below the threshold were shown to have significantly higher event-free survival than those with higher expression levels (log-rank p = 0.04, Fig. 2).

There was no significant difference in survival between heart recipients with miR-339 expression above and below the threshold (log-rank p = 0.34).

The relative risk of detecting graft myocardial fibrosis in heart recipients with miR-27 expression above the threshold was RR = 1.5 + 0.157 [95% CI 1.104–2.039], p = 0.009; for recipients with miR-339 expression above threshold, the risk of myocardial fibrosis was RR = 1.31 + 0.130 [95% CI 1.018–1.692], p = 0.036.

When miR-27 and miR-339 expression levels were simultaneously high (above threshold), the risk of developing graft heart myocardial fibrosis increased 1.93-fold (RR = 1.93 + 0.245 [95% CI 1.191–3.111], p = 0.007).

Results obtained indicate that miR-27 and miR-339 expressions are associated with development of myocardial fibrosis in transplanted heart, but the practical value of the tests is limited by their lack of sensitivity (not more than 60%). Therefore, a study was undertaken to investigate the combination of microRNA and galectin-3, a proteomic biomarker with proven efficacy in myocardial fibrosis.

Serum galectin-3 levels in recipients with myocardial fibrosis were significantly higher than those in recipients without fibrosis (p = 0.009, Fig. 3).

The diagnostic significance of galectin-3 in identifying patients with fibrosis was assessed. Fig. 4 shows the ROC curve of galectin-3 levels in heart recipients with graft myocardial fibrosis.

The area under the ROC curve for galectin-3 was 0.73 ± 0.077 [95% CI 0.574–0.879] and was significantly different from 0.5 (p = 0.004).

The highest Youden index was used to determine the diagnostically significantly galectin-3 threshold with respect to detection of myocardial fibrosis in the transplanted heart, which was 21.66 ng/mL; The relative risk of detecting graft myocardial fibrosis in recipients with galectin-3 levels above the threshold was RR = 1.46 + 0.157 [95% CI 1.071–1.978] (p = 0.016) with sensitivity and specificity of 60.9% and 78.6% respectively.

A comparative analysis of the survival of heart recipients with galectin-3 levels above and below the calculated thresholds showed a significant difference (Fig. 5).

In the group of patients with galectin-3 levels below 21.66 ng/mL, event-free survival was significantly higher than in recipients with galectin-3 levels higher than that (log-rank p = 0.003).

Evaluation of the diagnostic characteristics of tests involving combined measurement of miR-27 expression and galectin-3 levels showed the following results: with simultaneous expression level of miR-27 and galectin-3 levels above the calculated thresholds, the risk of myocardial fibrosis in the transplanted heart increases up to 2.7-fold (RR = 2.67 + 0.456 [95% CI 1.090–6.524], p = 0.032 (Fig. 6).

When miR-339 expression levels and serum galectin-3 levels exceeded their threshold at the same time, the risk of developing myocardial fibrosis increased by up to 2-fold (RR = 2.00 + 0.316 [95% CI 1.076–3.717], p = 0.028) as compared to individual tests.

Table 2 presents the main diagnostic characteristics of miR-27, miR-339, galectin-3 and their combinations in relation to development of myocardial fibrosis in the transplanted heart.

The combined measurement of miR-27 expression levels and serum galectin-3 levels in heart recipients has the best diagnostic characteristics in detecting myocardial fibrosis in transplanted hearts.

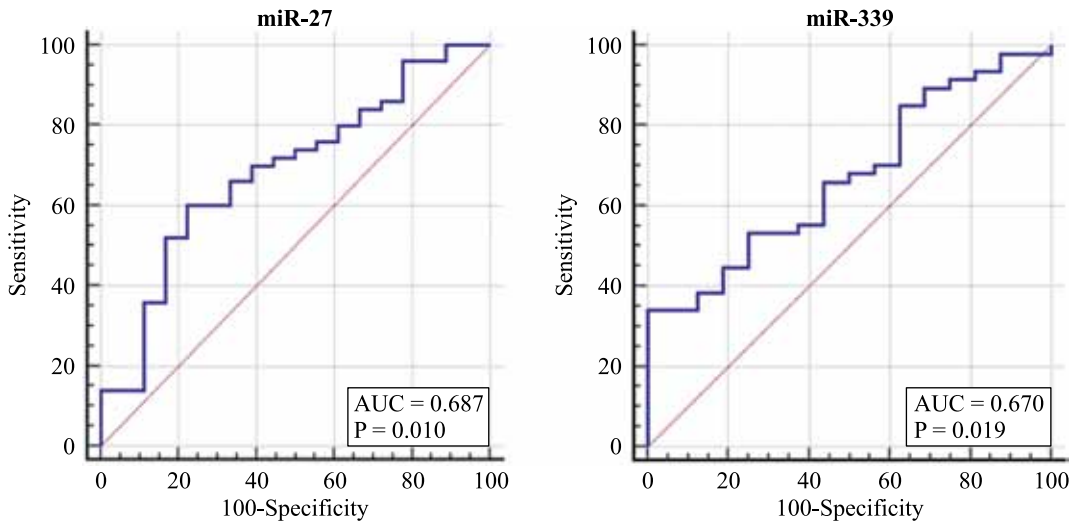

Fig. 1. ROC curves of miR-27 and miR-339 in heart recipients with graft myocardial fibrosis

DISCUSSION

Myocardial fibrosis plays an important role in the development of subclinical post-transplant heart failure. The formation of fibrous tissue in the intercellular space promotes structural and functional graft remodelling. Pathological factors such as arterial hypertension, acute

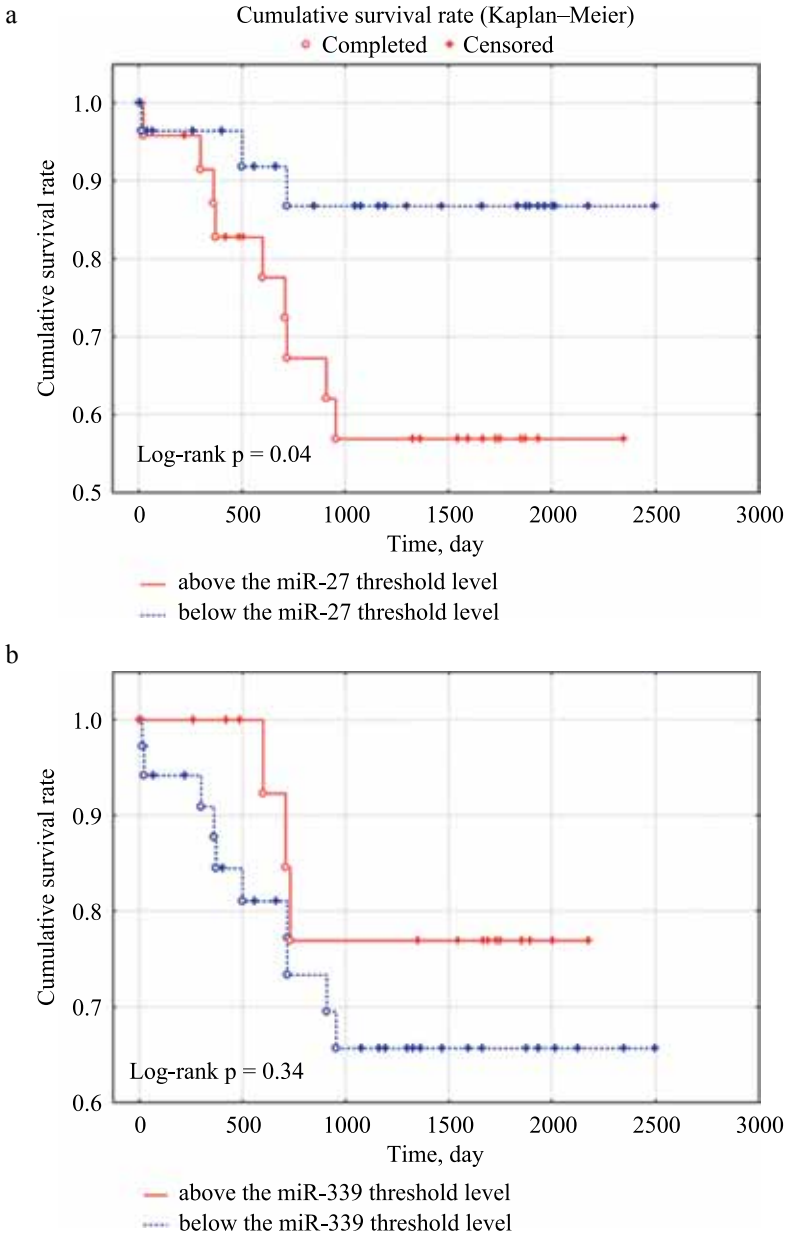

Fig. 2. Survival curves without adverse events in heart recipients as a function of miR-27 (a) and miR-339 (b) expression levels

Table 2

Diagnostic characteristics of miR-27, miR-339, and galectin-3 in relation to detection of myocardial fibrosis in heart recipients at levels above thresholds

Tests	Sensitivity	Specificity	PPV	NPV
miR-27	60.0%	77.8%	88.2%	41.2%
miR-339	36.2%	87.5%	89.5%	31.8%
galectin-3	60.9%	78.6%	90.3%	37.9%
miR-27 + miR-339	52.4%	91.7%	91.7%	52.4%
miR-27 + galectin-3	66.7%	100.0%	100.0%	62.5%
miR-339 + galectin-3	54.5%	100.0%	100.0%	50.0%

PPV – positive predictive value of the test; NPV – negative predictive value of the test.

rejection and graft vasculopathy lead to myocardial fibrosis in the transplanted heart [9, 10].

Myocardial fibrosis is verified by morphological analysis of myocardial tissue obtained during EMB, and its quantitative characterisation is performed by determining the collagen formation index – collagen volume fraction (CVF). CVF makes it possible to estimate the content and ratio of type I and type III collagen in the myocardium. However, the diagnostic value of this analysis can be significantly limited by possible errors when taking the examined biopsy material due to uneven distribution of collagen in tissues [11].

Over the past decade, the understanding of how fibrosis develops has significantly expanded. Fibroblasts play a key role in maintenance of extracellular matrix, they also regulate collagen synthesis and degradation. Transforming growth factor TGF-β1 and angiotensin II are the best known profibrogenic factors. Response to the action of angiotensin is manifested in the form of expression of galectin-3, an active stimulator of fibroblast proliferation. Similar stimulation of fibroblasts is observed in the activation of the immune system caused by oxidative stress or due to mechanical damage to the atria [12].

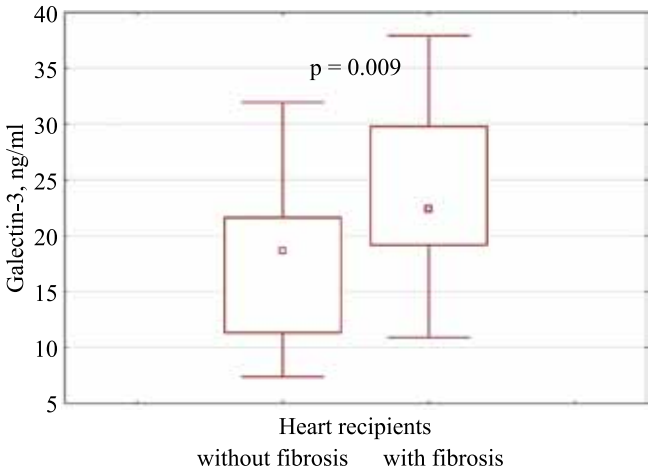

Fig. 3. Galectin-3 serum concentrations in heart recipients with and without myocardial fibrosis

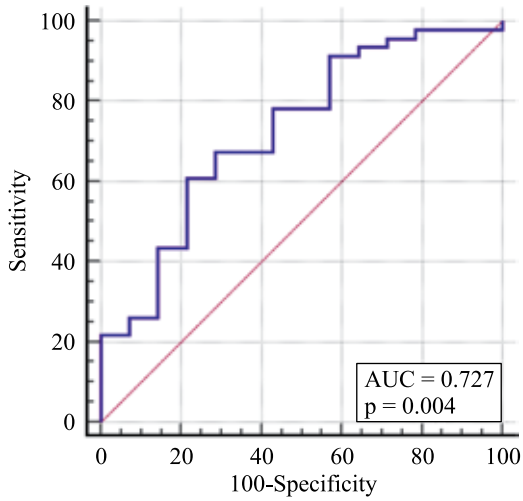

Fig. 4. ROC curve of galectin-3 serum concentrations in heart recipients with myocardial fibrosis

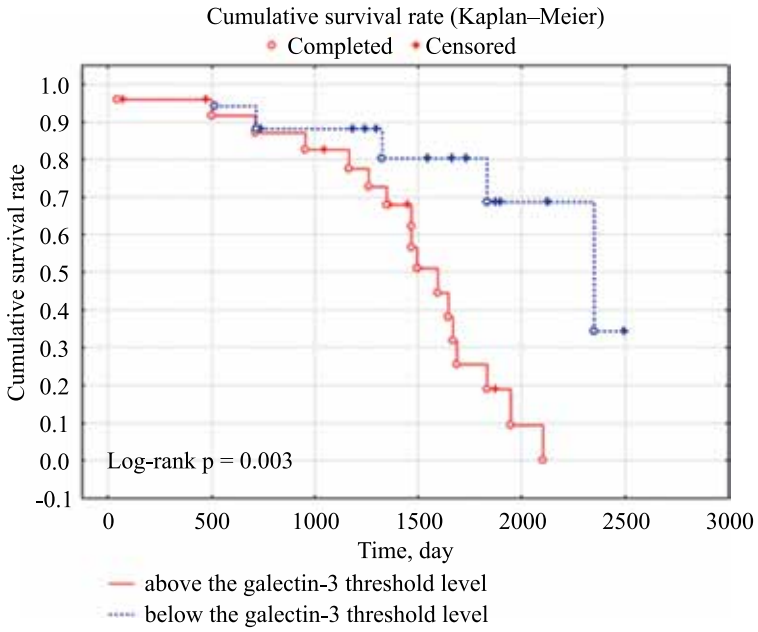

Fig. 5. Survival curves without adverse events in heart recipients with galectin-3 serum concentrations above and below threshold levels

Circulating microRNAs play an important role in heart remodelling and other important biological processes. Huang YM et al. evaluated the diagnostic potential of a number of microRNAs in relation to the development and course of heart failure in patients with coronary heart disease and dilated cardiomyopathy [4].

The function of miRNA molecules is associated with regulation of gene expression, namely with their inhibitory effect on RNA at the level of transcription processes. The regulatory role of miR-30 and -133a in myocardial fibrosis mechanisms through inhibition of connective tissue growth factor has been shown [13]. In experiments on mice, it was found that increased miR-133 expression leads to decreased collagen synthesis, and consequently decreased myocardial fibrosis, whereas absence of miR-133 is associated with high susceptibility to heart failure (HF) and fibrosis. In addition, miR-21 is involved in regulation through one of the profibrotic pathways and has a protective effect against oxidative stress. Finally, miR-29 is associated with deposition of collagen types I and III. Increased miR-29 leads to decreased synthesis of these proteins and vice versa.

Our findings with respect to the association of miR-27 and miR-339 with fibrosis are consistent with reports from foreign authors. There is evidence of the inhibitory effect of miR-27 and miR-101 on the development of fibrotic processes in heart and other organs [14–16]. A number of studies have noted the involvement of miR-27 as an inhibitor of myocardial inflammatory responses [17], and that miR-27b, as a member of microRNA-27 family, can play antifibrotic role in left atrium and be considered as a novel therapeutic target for heart failure. The mechanism of antifibrotic action of miR-27 is attributed to its inhibitory effect on TGF-β [18]. Meanwhile, there is little evidence on the profibrotic effect of miR-27 through inhibition of the synthesis of transcriptional protein FBW7 [19]. It was found that miR-27 is important in the development of atherosclerosis: by suppressing lipoprotein lipase-induced lipid accumulation and inflammatory response, miR-27 reduces progression of atherosclerosis [20]. Cruz L.O. et al. reported that miR-27 can disrupt Treg cell differentiation, thereby reducing Treg-mediated immunological tolerance [21]. Along with that, recent studies have shown that miR-339 is involved as an inhibitor of cell proliferation [22, 23]. In coronary heart disease, miR-339 is able to increase oxidative stress by inhibiting the Nrf2/FOXO3 signaling pathway via specific protein Sirt2 [24]. A study by Chen J. et al. showed that miR-339 suppresses pulmonary artery smooth muscle cell proliferation through inhibition of FGF signaling pathway [25]. Our results suggest that heart recipients with the development of myocardial fibrotic processes have significantly higher expression levels of miR-27 and -339.

Another new biomarker for the development of severe heart failure is galectin-3, a member of the lectin family. It plays an important role in regulation of inflammation, immune response and nerve tissue degeneration, and has also been identified as a profibrogenic factor. Galectin-3 is secreted into the extracellular space at the site of injury and activates previously resting fibroblasts [26]. Coromilas et al. found that increased galectin-3 levels were associated with development of heart failure, whereas lower galectin-3 levels were associated with lower severity of the disease [27]. The prognostic significance of galectin-3 in the risk of adverse events in patients with heart failure has thus been demonstrated.

We have previously shown the diagnostic potential of galectin-3 in relation to myocardial fibrosis in a transplanted heart [28, 29]. In the present study, we have evaluated variants of tests of its combination with microRNAs miR-27 and miR-339, potentially significant in the development of heart failure [29].

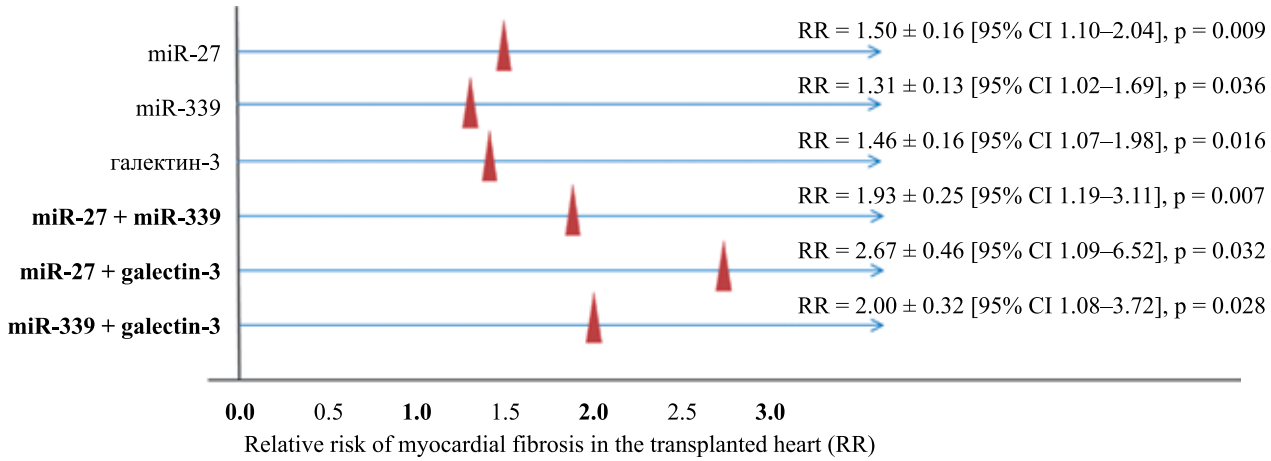

Fig. 6. Relative risks of myocardial fibrosis in heart recipients with miR-27, miR-339 expression levels and galectin-3 serum concentrations above threshold levels, and when they are determined together

The best diagnostic characteristics in detecting myocardial fibrosis in transplanted hearts was found to be the combined test for miR-27 expression level and serum galectin-3 levels in heart recipients.

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The authors declare no conflict of interest.

REFERENCES

1. *Crespo-Leiro MG, Barge-Caballero G, Couto-Mallon D.* Noninvasive monitoring of acute and chronic rejection in heart transplantation. *Curr Opin Cardiol.* 2017; 32 (3): 308–315.
2. *Shevchenko AO, Nikitina EA, Koloskova NN, Shevchenko OP, Gautier SV.* Kontroliruemaja arterial'naja gipertenzija i vyzhivaemost' bez nezhelatel'nyh sobytij u recipientov serdca. *Kardiovaskuljarnaja terapija i profilaktika*. 2018; 17 (4): 4–11. [In Russ, English abstract].
3. *Shah P, Bristow MR, Port JD.* MicroRNAs in Heart Failure, Cardiac Transplantation, and Myocardial Recovery: Biomarkers with Therapeutic Potential. *Curr Heart Fail Rep.* 2017; 14 (6): 454–464.
4. *Huang YM, Li WW, Wu J, Han M, Li BH.* The diagnostic value of circulating microRNAs in heart failure. *Exp Ther Med.* 2019; 17 (3): 1985–2003.
5. *Duong Van Huyen JP, Tible M, Gay A.* MicroRNAs as non-invasive biomarkers of heart transplant rejection. *European Heart Journal*. 2014; 35 (45): 3194–3202.
6. *Farid WRR, Pan Q, Van der Meer AJP.* Hepatocyte-derived microRNAs as serum biomarkers of hepatic injury and rejection after liver transplantation. *Liver Transplantation*. 2012; 18 (3): 290–297.
7. *Livak KJ, Schmittgen TD.* Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001; 25 (4): 402–408.
8. *Hughes G.* Youden's Index and the Weight of Evidence Revisited. *Methods Inf Med.* 2015; 54 (6): 576–577.
9. *Drapkina OM, Drapkina YuS.* Fibrosis and renin-angiotensin-aldosterone system activity. Reality and future prospects. *Arterial Hypertension*. 2012; 18 (5): 449–458.
10. *Khush K, Zarafshar S.* Molecular Diagnostic Testing in Cardiac Transplantation. *Curr Cardiol Rep.* 2017; 19 (11): 118.
11. *Stehlik J, Starling RC, Movsesian MA et al.* Utility of long-term surveillance endomyocardial biopsy: a multi-institutional analysis. *J Heart Lung Transplant.* 2006; 25 (12): 1402–1409.
12. *Miklishanskaya SV, Mazur NA, Shestakova NV.* Mekhanizmy formirovaniya miokardial'nogo fibroza v norme i pri nekotoryh serdechno-sosudistyh zabolevaniyah. metody ego diagnostiki. *Medicinskij sovet*. 2017; 12: 75–81.
13. *Dzeshka MS, Lip GY, Snezhitskiy V, Shantsila E.* Cardiac Fibrosis in Patients With Atrial Fibrillation. *JACC*. 2015; 66 (8): 943–959.
14. *Li X, Zhang S, Wa M et al.* MicroRNA-101 Protects Against Cardiac Remodeling Following Myocardial Infarction via Downregulation of Runt-Related Transcription Factor 1. *J Am Heart Assoc.* 2019; 8 (23): e013112.
15. *Huang C, Xiao X, Yang Y et al.* MicroRNA-101 attenuates pulmonary fibrosis by inhibiting fibroblast proliferation and activation. *J Biol Chem*. 2017; 292 (40): 16420–16439.
16. *Meroni M, Longo M, Erconi V et al.* Mir-101-3p Downregulation Promotes Fibrogenesis by Facilitating Hepatic Stellate Cell Transdifferentiation During Insulin Resistance. *Nutrients*. 2019; 11 (11): 2597.
17. *Zhang XL, An BF, Zhang GC*. MiR-27 alleviates myocardial cell damage induced by hypoxia/reoxygenation via targeting TGFBR1 and inhibiting NF-κB pathway. *Kaohsiung J Med Sci.* 2019; 35 (10): 607–614.
18. *Wang Y, Cai H, Li H, Gao Z, Song K.* Atrial overexpression of microRNA-27b attenuates angiotensin II-induced atrial fibrosis and fibrillation by targeting ALK5. *Hum Cell*. 2018; 31 (3): 251–260.
19. *Fu Q, Lu Z, Fu X et al.* MicroRNA 27b promotes cardiac fibrosis by targeting the FBW7/Snail pathway. *Aging (Albany NY)*. 2019; 11 (24): 11865–11879.
20. *Xie W, Li L, Zhang M et al.* MicroRNA-27 Prevents Atherosclerosis by Suppressing Lipoprotein Lipase-Induced Lipid Accumulation and Inflammatory Response in Apolipoprotein E Knockout Mice. *PLoS One*. 2016; 11 (6): e0157085.
21. *Cruz LO, Hashemifar SS, Wu CJ et al.* Excessive expression of miR-27 impairs Treg-mediated immunological tolerance. *J Clin Invest*. 2017; 127 (2): 530–542.
22. *Zeng H, Zheng J, Wen S et al.* MicroRNA-339 inhibits human hepatocellular carcinoma proliferation and invasion via targeting ZNF689. *Drug Des Devel Ther*. 2019; 13: 435–445.
23. *Derda AA, Pfanne A, Bär C et al.* Blood-based microRNA profiling in patients with cardiac amyloidosis. *PLoS One*. 2018; 3 (10): e0204235.
24. *Shi L, Zhang Y, Zhang J et al.* MiR-339 is a potential biomarker of coronary heart disease to aggravate oxidative stress through Nrf2/FOXO3 targeting Sirt2. *Ann Palliat Med*. 2021; 10 (3): 2596–2609
25. *Chen J, Cui X, Li L et al.* MiR-339 inhibits proliferation of pulmonary artery smooth muscle cell by targeting FGF signaling. *Physiol Rep*. 2017; 5 (18): e13441.
26. *Grupper A, Nativi-Nativi J, Maleszewski JJ et al.* Circulating galectin-3 levels are persistently elevated after heart transplantation and are associated with renal dysfunction. *JACC: Heart Failure.* 2016; 4: 847–856.
27. *Coromilas E, Que-Xu E, Moore D et al.* Dynamics and prognostic role of galectin-3 in patients with advanced heart failure, during left ventricular assist device support and following heart transplantation. *BMC Cardiovasc Disord*. 2016; 16: 138–148.
28. *Shevchenko OP, Ulybysheva AA, Gichkun OE et al.* Galectin-3 in rejection and fibrosis of the transplanted heart. *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (3): 62–68. [In Russ, English abstract]. doi: 10.15825/1995-1191-2019-3-62-68.
29. *Velikij D.A., Gichkun O.E., Shevchenko A.O.* MikroRNK: rol' v razvitii serdechno-sosudistyh zabolevanij, perspektivy klinicheskogo primeneniya. *Klinicheskaya laboratornaya diagnostika*. 2018; 63 (7): 403–409.

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