Expression of miR-146a-5p and miR-155-5p in Myocardial Tissue: A Shared NF-κB-Related Inflammatory Signature Across HFpEF and HFrEF Phenotypes

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Abstract **— Background: Chronic heart failure (CHF) is a growing global health burden, with heart failure with preserved ejection fraction (HFpEF) increasingly outpacing heart failure with reduced ejection fraction (HFrEF) in prevalence, while conventional biomarkers show limited diagnostic value in HFpEF. MicroRNA-146a-5p and microRNA-155-5p are key regulators of the NF-κB-dependent inflammatory axis implicated in myocardial remodeling, yet their comparative expression across CHF phenotypes remains unexplored. This study aimed to evaluate and compare the expression of miR-146a-5p and miR-155-5p in heart tissue from patients with HFpEF and HFrEF. Methods: Heart tissue specimens were obtained from 44 patients with chronic heart failure (22 with HFpEF and 22 with HFrEF). Reverse transcription and quantitative real-time PCR were performed using TaqMan MicroRNA assays, with RNU6, RNU44, and RNU48 used as reference RNAs (inter-assay variability <5%). Relative expression was calculated using the $2^{-}\Delta\Delta Ct$ method. Normality of distribution was assessed via the Shapiro-Wilk test; between-group differences were evaluated using one-way ANOVA and multivariate ANOVA (MANOVA), with significance set at $p \leqslant 0.05$. Results: Both miR-146a-5p and miR-155-5p were significantly upregulated relative to reference RNAs in both HFpEF and HFrEF groups (2.40 ± 0.08-fold and 2.48 ± 0.08-fold for miR-146a-5p; 1.80 ± 0.10-fold and 1.86 ± 0.10-fold for miR-155-5p, respectively; $p < 0.001$). However, no statistically significant differences in expression levels of either microRNA were found between the HFpEF and HFrEF groups (ANOVA, $p > 0.05$ for both). Multivariate analysis confirmed the absence of a significant combined between-group difference (Wilks' $\lambda = 0.980$, $F(2,41) = 0.421$, $p > 0.05$). Conclusion: miR-146a-5p and miR-155-5p are consistently upregulated components of the NF-κB inflammatory axis in chronic heart failure regardless of ejection fraction phenotype, but their expression levels do not differentiate HFpEF from HFrEF in this cohort. These findings suggest a shared inflammatory signature across CHF phenotypes rather than a phenotype-specific one, warranting validation in larger, multicenter cohorts.**

Keywords **— chronic heart failure, HFpEF, HFrEF, miR-146a-5p, miR-155-5p, NF-κB, inflammation, biomarker.**

I. Introduction

Chronic heart failure (CHF) is one of the most significant problems in modern cardiology. According to current estimates, the number of people with this syndrome worldwide exceeds 55 million. Moreover, the prevalence continues to grow against the backdrop of an aging population and an increase in the number of concomitant diseases [1].

It is important that the structure of CHF according to the phenotypes of the ejection fraction (EF) is changing. The prevalence of CHF with reduced ejection fraction (HFrEF) remains stable or decreases. In contrast, the prevalence of CHF with preserved ejection fraction (HFpEF) is increasing and may become the dominant phenotype in the future. Traditional biomarkers, such as natriuretic peptides and cardiac troponins, have significantly lower diagnostic and prognostic value specifically in HFpEF. Therefore, the search for non-coding RNA biomarkers as an innovative approach to stratifying patients into risk groups is becoming relevant [2].

The study of miR-146a and miR-155 is of particular importance. These are two key regulators of the NF-κB axis, which underlies the pro-inflammatory remodeling of the myocardium in both phenotypes of CHF. MiR-146a, induced by pro-inflammatory cytokines interleukin-1β and tumor necrosis factor-α, suppresses the NF-κB signaling pathway. This leads to the suppression of inflammation. The reduction in the expression of miR-146a leads to sustained activation of NF- κB via the IRAK1 and TRAF6 proteins, thereby enhancing the inflammatory response [3]. The molecular mechanism of this interaction involves the transcription of miR-146a, which is induced in the myocardium precisely upon activation of NF-κB — a

transcription factor triggered by pro-inflammatory molecules such as TNF-α. This factor is closely linked to the pathogenesis of cardiac disorders. It has been experimentally established that miR-146a affects the components of the Toll-like receptor/NF-κB signaling pathway. It directly targets the key adapter molecules of this cascade, IRAK1 and TRAF6. This process has been demonstrated in models of ischemic-reperfusion myocardial injury and endotoxin-induced monocyte tolerance [4]. The functional significance of this mechanism has also been confirmed in the context of sepsis-induced cardiac dysfunction, where the overexpression of miR-146a mitigated myocardial damage by negatively regulating NF-κB activation and reducing the production of pro-inflammatory cytokines through its effect on ErbB4 [5].

Unlike miR-146a, which plays a cardioprotective role, miR-155 predominantly exhibits a pro-inflammatory effect within the same regulatory axis. It has been established that miR-155 promotes the activation of pro-inflammatory type 1 (M1) macrophages, the production of reactive oxygen species, and the secretion of IL-1β and TNF-α. At the same time, the inflammatory response was enhanced. The expression of miR-155 is suppressed by the anti-inflammatory interleukin-10 [6]. Studies show that in models of ischemia-reperfusion injury to the myocardium, miR-155-5p promoted the polarization of macrophages towards a pro-inflammatory M1 phenotype due to the activation of the JAK2/STAT1 signaling pathway. The inflammatory response was thereby exacerbated. Therefore, the inhibition of this microRNA is considered a promising therapeutic approach to modulating macrophage inflammation [7]. The relationship between miR-155 and the NF-κB axis has also been demonstrated in the context of atherosclerotic damage to the vascular wall. It has been proven that miR-155 can enhance pro-inflammatory processes and atherosclerotic damage through the activation of STAT3 and NF-κB via targeting the cytokine signaling suppressor SOCS1 [8].

Thus, miR-146a and miR-155 act as divergent but functionally interconnected nodes regulating the NF-κB axis in the myocardium. The former provides a negative feedback mechanism that limits the pro-inflammatory response and the IRAK1/TRAF6-dependent signaling mediated by it. The latter, by contrast, supports the pro-inflammatory polarization of macrophages and enhances NF-κB-dependent cytokine transcription. This dichotomy is examined in current studies specifically in relation to the pathogenesis of HFpEF. The role of miR-146a, miRNA-155, and miR-223 in modulating myocardial inflammation is recognized as extremely significant for the progression of this particular phenotype of heart failure, in which fibro-inflammatory myocardial remodeling plays a key pathogenetic role [9]. The multidirectional nature of the regulation of NF-κB-dependent inflammation by these two microRNAs and their dysregulation in HFrEF and HFpEF make further study of miR-146a and miR-155 in various phenotypes of CHF relevant. This will deepen the understanding of the molecular mechanisms of inflammatory myocardial remodeling and will make it possible to assess their prognostic and therapeutic potential.

II. Material and Methods

A. Samples and Data Collection

Specimens from heart tissues were obtained from 44 individuals diagnosed with chronic heart failure who underwent treatment at the Clinic of Bashkir State Medical University over the period spanning from 2024 to 2026.

Written informed consent for the collection of biological liquids and tissues and participation in the scientific research was received from each patient. The study protocol received approval from the Research Ethics Committee of the Institute of Biochemistry and Genetics, a subdivision of the Ufa Federal Research Center of the Russian Academy of Sciences. The study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki by the World Association.

B. miRNA Extraction, Reverse Transcription, Expression Analysis of miRNA-146a and miRNA-155

The reverse transcription of miRNAs was conducted to examine their varying expression levels. This process utilized the TaqMan®® MicroRNA Reverse Transcription Kit, provided by Applied Biosystems (Waltham, MA, USA). The reaction mixture comprised 5 µL of total RNA, 7 µL of a master mixture containing reverse transcriptase, dNTPs, and other essential components, along with 3 µL of the 5X OT primer designed specifically for the targeted miRNA gene. The reverse transcription reaction was carried out according to the following conditions: 16 °C for 30 min, 42 °C for 30 min, 85 °C for 5 min, and finally held at 4 °C indefinitely. For real-time quantitative PCR analysis, the TaqMan MicroRNA Assays kit from

Applied Biosystems and the CFX96™ Real-Time PCR Product Detection System from BioRad (Hercules, CA, USA) were used. All samples were analyzed in triplicate, and all experiments were performed at least three times.

C. Statistical Analysis

The $2^{-\Delta\Delta Ct}$ method [10] was used to quantitatively assess gene expression. Statistical data processing was carried out using specialized software, CFX Maestro version 2.3 (Bio-Rad, USA), which is designed for analyzing and visualizing PCR results. During the analysis, the normality of data distribution was checked (Shapiro-Wilk test), the significance of differences in the expression of microRNAs between groups was assessed (ANOVA and MANOVA calculations), and the reliability of the obtained values was evaluated. At $p \leqslant 0.05$, the differences were considered significant. RStudio software was used to correlate the expression levels of microRNA with each other and with the stages of sKPCK, and to visualize the results.

III. Results and Discussion

We assessed the expression of microRNAs miR-146a-5p and miR-155-5p in cardiac tissues from 22 patients with chronic heart failure with preserved left ventricular ejection fraction (HFpEF) and 22 patients with chronic heart failure with reduced left ventricular ejection fraction (HFrEF). The following RNAs were used as references: RNU6, RNU44, RNU48; the variability in their expression was less than 5 % according to the analysis results using the CFX Maestro software version 2.3.

During the study, we found that miR-146a-5p is statistically significantly highly expressed in the cardiac tissues of patients with HFrEF and HFpEF compared to the reference. Its expression levels were increased by 2.40 ± 0.08 times ($p < 0.001$) and 2.48 ± 0.08 times ($p < 0.001$) in the HFpEF and HFrEF groups, respectively (Fig. 1). The results we obtained do not contradict other studies. Cardiomyocytes, the cells of the heart muscle, are saturated with miR-146a, which regulates their survival and the independent regulation of the inflammatory process [11]. Information about the presumed influence of miR-146a-5p on the development of chronic heart failure and left ventricular remodeling is found in scientific articles. In the study by Xiao S. et al. It was noted that in the postoperative period, the level of miR-146a decreased, but a week later, the expression of the microRNA increased again. This effect could have been caused by a combination of factors: successful restoration of blood flow in cardiomyocytes and the use of medications [12]. It is also known that miR-146a is involved in the development of inflammation and fibrosis, which is associated with left ventricular remodeling. Induced miR-146a targets inflammatory molecules, thereby reducing inflammation. [11, 13, 14]. There are also conflicting data on the role of miR-146a. Das K. et al. presented data that miR-146a deficiency leads to increased expression of proinflammatory cytokines IL-1b, IL-18, Micu M.A. et al. came to a similar conclusion that with a decrease in miR-146a levels, post-transcriptional suppression of NF-kB pathway components does not occur with HFpEF [9, 15, 16]. It is believed to perform a protective function in relation to cardiomyocytes, extending their lifespan and protecting them from oxidative stress [11, 17, 18].

We conducted a similar analysis for another pro-inflammatory microRNA — miR-155-5p. Its expression exceeded the value of reference microRNAs by 1.80 ± 0.10 ($p < 0.001$) in patients with HFpEF and by 1.86 ± 0.10 ($p < 0.001$) in patients with HFrEF (Fig. 2). Micu M.A. et al. In their work, they demonstrated that miR-155 performs several functions in cardiac pathology. A number of studies have shown that an increase in its expression can lead to HFpEF via an inflammatory response. MiR-155 protects cardiomyocytes from apoptosis, modulates the density of myofibroblasts, has a great effect on T-cell differentiation and maturation of dendritic and B cells [9, 16, 19, 20, 21]. MiR-155 affects the polarization of type 1 macrophages (M1), which leads to the appearance of pro-inflammatory derivatives and oxidative stress in the context of myocardial inflammation. Thus, targeted suppression of miR-155 inhibits M1 polarization, reduces the concentration of pro-inflammatory factors, and potentially mitigates the chronic inflammation characteristic of HFpEF [6, 9, 22].

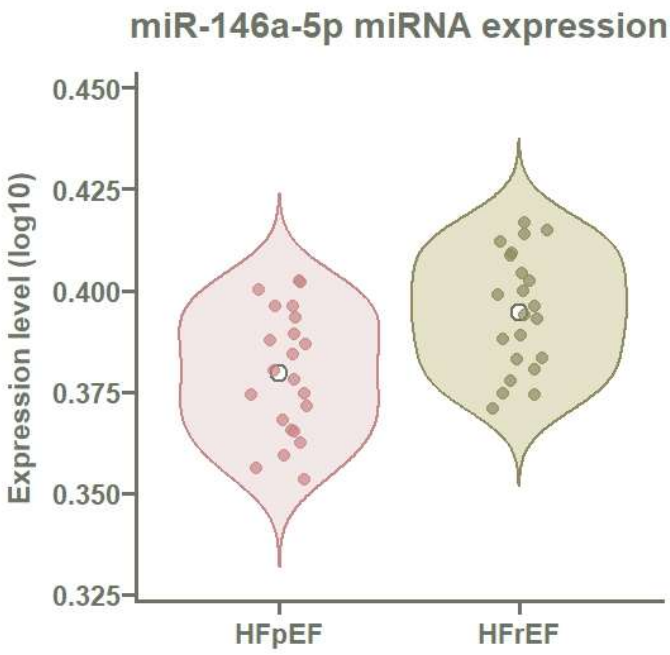

Fig. 1. Comparative analysis of miR-146a-5p expression in HFpEF and HFrEF heart tissues relative to the reference

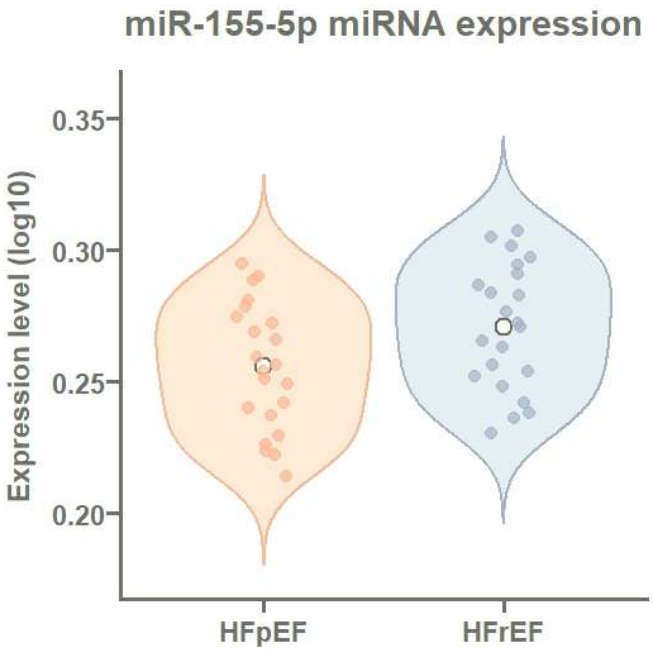

Fig. 2. Comparative analysis of miR-155-5p expression in HFpEF and HFrEF heart tissues relative to the reference miRNAs

The expression levels of miR-146a-5p and miR-155-5p in cardiac tissues in the groups of patients with HFpEF and HFrEF did not differ statistically significantly when compared to each other (Table 1, Fig. 3, Fig. 4).

TABLE I
Comparison of expression levels of miR-146a-5p and miR-155-5p in heart tissues, depending on the type of CHF

miRNA	**miRNAs expression levels**		**p**
	HFpEF	**HFrEF**	
miR-146a-5p	1,00 ± 0,04	1,04 ± 0,03	> 0,05
miR-155-5p	1,00 ± 0,06	1,04 ± 0,06	> 0,05

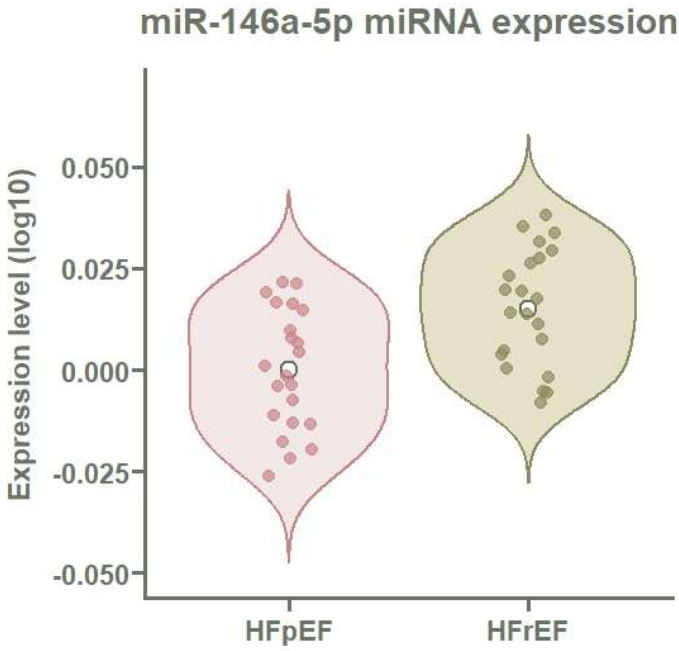

Fig. 3 Comparative analysis of miR-146a-5p expression levels in heart tissues between groups of patients with HFpEF and HFrEF

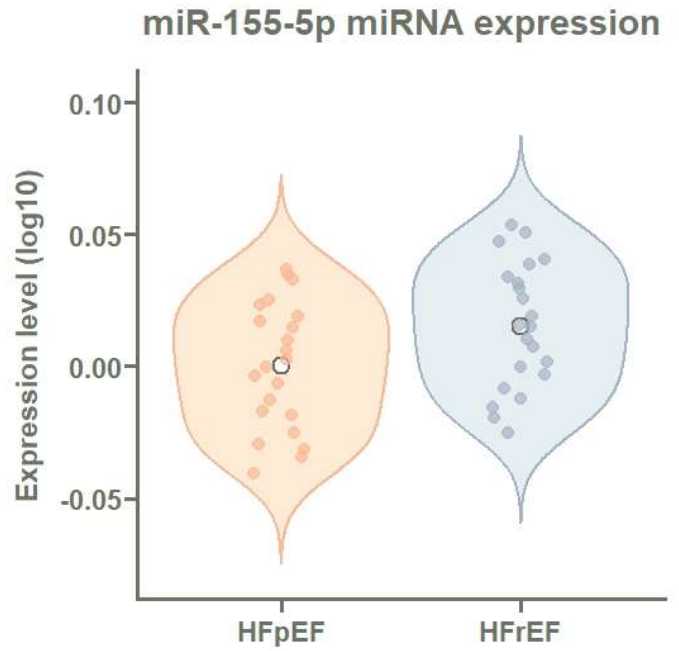

Fig. 4. Comparative analysis of miR-155-5p expression levels in heart tissues between groups of patients with HFpEF and HFrEF

To compare the expression levels of miR-146a-5p and miR-155-5p between the HFpEF and HFrEF groups, a one-way ANOVA analysis was used; the results showed no statistically significant differences for either microRNA. Multivariate analysis by MANOVA confirmed a statistically insignificant difference between HFpEF and HFrEF for miR-146a-5p and miR-155-5p as components of the NF-κB-dependent regulatory axis involved in controlling the inflammatory response (Wilkis's $\lambda = 0.980$; $F(2.41) = 0.421$; $p > 0.05$) (Table 2).

TABLE III
Comparison of expression levels of miR-146a-5p and miR-155-5p in heart tissues, depending on the type of CHF

Shapiro-Wilk's test						
miRNA		Group	n	W	p	Conclusion
miR-146a-5p		HFpEF	22	0,961	0,51	Normal
miR-146a-5p		HFrEF	22	0,963	0,55	Normal
miR-155-5p		HFpEF	22	0,954	0,37	Normal
miR-155-5p		HFrEF	22	0,953	0,36	Normal
ANOVA						
miRNA	Comparison	Model	F		df	p	Conclusion
miR-146a-5p	HFpEF vs. HFrEF	HFpEF vs. HFrEF	0,64		1,42	0,42	Not significant
miR-155-5p	HFpEF vs. HFrEF	HFpEF vs. HFrEF	0,22		1,42	0,63	Not significant
MANOVA						
Type	Comparison	Wilks' λ	F		df	p	Conclusion
miR-146a-5p & miR-155-5p	HFpEF vs. HFrEF	0,980	0,421		2,41	0,66	Not significant

Note: n – the number of samples; W – the statistics of the Shapiro-Wilk test; p – the level of significance; F – the Fisher criterion for ANOVA and MANOVA; df – the degree of freedom; Wilks' λ – the MANOVA indicator.

To date, we have not found any published data in the sources available to us regarding studies of the expression levels of miR-146a-5p and miR-155-5p in groups of patients with chronic heart failure with HFpEF and HFrEF.

IV. Conclusions

In this study, for the first time, a comparative assessment was carried out of the expression of miR-146a-5p and miR-155-5p — key regulators of the NF-κB-dependent inflammatory pathway — in the cardiac tissues of patients with two main phenotypes of chronic heart failure: HFpEF and HFrEF. Both microRNAs demonstrated a statistically significant increase in expression relative to reference RNAs in both groups.

($p < 0.001$), which confirms their involvement in the pro-inflammatory remodeling of the myocardium, regardless of the CHF phenotype. However, no statistically significant intergroup differences in the expression levels of miR-146a-5p and miR-155-5p were detected between HFpEF and HFrEF (ANOVA, $p > 0.05$; MANOVA, Wilks' $\lambda = 0.980$, $p > 0.05$), which indicates a similar degree of activation of this inflammatory pathway in both CHF phenotypes, despite the differences in their pathophysiology and clinical course. The data obtained suggest that miR-146a-5p and miR-155-5p do not possess sufficient discriminatory power to distinguish patients by CHF phenotype and likely reflect a general, rather than phenotype-specific, component of myocardial inflammation. Given the limited sample size ($n = 44$) and the lack of comparable literature data, the results obtained require confirmation in larger and multicenter cohorts, as well as additional study in combination with other markers of inflammation and fibrosis to assess their combined diagnostic and prognostic value in various phenotypes of chronic heart failure.

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Acknowledgment

The authors declare no conflict of interest.

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