

Beta-adrenergic reactivity of erythrocytes and the progression of heart failure in patients after myocardial infarction

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Aim. To identify the associations between beta-adrenergic reactivity of erythrocytes and the progression of heart failure (HF) in patients after myocardial infarction (MI).

Material and methods. The study included 50 patients with HF and history of MI 6 months ago. To determine the level of sympathoadrenal system activity, we analyzed beta-adrenergic reactivity by changing the osmotic resistance of erythrocytes by use of adrenoceptor blocking agent.

Results. The frequency of HF progression after index MI was 26% (n=13). All patients were divided into 2 groups depending on the presence/absence of HF progression in the postinfarction period.

When determining beta-adrenergic reactivity, it was found that patients with HF progression compared with patients without it had the higher level of beta-adrenergic reactivity of membrane (β -ARM) of erythrocytes: 58,8 (50,9; 78,0) CU and 46,8 (38,0; 66,3) CU, $p=0,025$). A ROC analysis made it possible to establish the β -ARM level $\geq 49,53$ CU a cut-off point, which can be considered as a marker of HF progression in patients after MI (sensitivity 92,3%, specificity 62,2%). This level of β -ARM is associated with a more than five-fold increase of HF progression risk in patients after MI (OR 5,48; 95% CI 1,28-23,37; $p=0,024$).

Conclusion. In patients with HF and MI history, there is a decrease in the adrenergic reactivity of erythrocyte cell

membrane, which is reflected by an increase of β -ARM above normal range of 20 CU. At the same time, β -ARM in patients with HF progression compared with patients without it is significantly increased. Established cut-off point of β -ARM ($\geq 49,53$ CU) allows predicting the HF progression with high sensitivity and specificity.

Key words: adrenergic reactivity, heart failure, myocardial infarction, sympathoadrenal system.

Relationships and Activities: not.

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Heart failure (HF) is a prime medical and socio-economic problem, the relevance of which increases with age [1, 2]. Follow-up of a representative sample of the European part of the Russian Federation (according to the EPOCHA-CHF study) revealed a significant increase in the number of patients with HF over the past 16 years from 4,9 to 8,5%, and the number of patients with severe class III-IV HF increased from 1,8 to 3,1% [1]. According to foreign studies, HF incidence in the population is 2-3%, increasing with age to 7% [3].

One of the main causes of HF is coronary artery disease (CAD). CAD, including myocardial infarction (MI), is identified in 60-70% of patients with HF [1, 4]. An important aspect is the study of the long-term prognosis of patients after MI. This is especially urgent due to big number of available diagnostic and therapeutic approaches to the management of patients with acute coronary pathology [5].

Activation of the sympathoadrenal system (SAS) plays an important role in the pathogenesis of HF and MI [6-8]. Increased sympathetic tone makes a significant contribution to the pathogenesis of HF and affects the course and prognosis of the disease [6, 9, 10]. According to published data, high norepinephrine levels are observed in patients with HF, especially in the advanced stages [9].

At the beginning of this century, R. I. Struk and I. G. Dlusskaya developed an original method for studying the functional state of SAS, based on assessing the degree of adrenergic receptor desensitization to the long-term or regularly occurring effects of high catecholamine levels [11]. So, this is an express method for determining adrenergic reactivity for assessing the destructive effect of catecholamines on membrane cell structures. Inhibition of erythrocyte osmolysis depends on the number of functionally active β -adrenergic receptors on the cell surface and indicates their adrenergic reactivity [11]. In recent decades, experimental studies have established that erythrocytes demonstrates the general patterns of changes in membrane and cell structures under the action of catecholamines and can reflect systemic manifestations of SAS activity [12].

With long-term pronounced catecholamine stimulation, the number of adrenergic receptors on the erythrocyte membrane decreases and their functional state changes as a manifestation of the desensitization of the cell membrane [13]. This fact is a reflection of the single feedback principle of the neuroendocrine system, which demonstrates the inverse relationship between the blood catecholamine levels and the number of cell membrane receptors for them. Accordingly, with an increase of SAS mediators in the blood, the adrenergic receptors of erythrocyte cell membranes desensitize, and beta-adrenergic reactiv-

ity of membrane (β -ARM) of erythrocytes increases, while the actual adrenergic reactivity decreases. Conversely, with a decrease in the mediator concentration, β -ARM values decrease, and adrenergic reactivity increases [11]. Thus, the study of the functional state of β -adrenergic receptors to determine the β -AWP value in cardiovascular diseases is an urgent direction aimed at early diagnosis and prognostication. This is also can be used in actual clinical practice due to the high method availability.

Currently, there are few publications in Russia devoted to the problem of studying the functional state of SAS using the presented method in HF, and there are practically no studies evaluating adrenergic reactivity in HF after MI. In this regard, the aim of this study was to identify the association of erythrocyte beta-adrenergic reactivity with progression of HF in patients after MI.

Material and methods

The study included 50 patients (80% men) 6 months after MI with NYHA class I-III HF. There were following exclusion criteria: thyrotoxicosis; cancer; mental disorder; autoimmune disease; end-stage liver and kidney disease; acute or exacerbation of chronic infectious diseases; decompensated diabetes; valvular heart disease; NYHA class IV HF.

The data collection on the features of acute MI was carried out based on research and information database "Acute myocardial infarction register" of the Tomsk National Research Medical Center (Cardiology Research Institute). We also analyzed outpatient medical records, case histories, and discharge from them.

The mean age of patients at the inclusion time was $57,0 \pm 11,5$ years in the male cohort ($n=40$) and $72,1 \pm 10,2$ years in the female ($n=10$). Therefore, men were significantly younger than women ($p<0,001$, $t=-3,79$). The diagnosis of HF was established in accordance with Russian and European guidelines for the management of heart failure [1, 2]. The clinical condition of patients, in addition to determining the class of HF, was assessed using the clinical state scale (CSS) in V. Yu. Mareev's modification. We also analyzed the quality of life of patients by the EQ-5D-3L questionnaire, as well as the therapy taken by patients at the inclusion time.

At the time of inclusion in the study, all patients underwent beta-adrenergic reactivity analysis to determine the change in erythrocyte osmotic resistance by use of adrenoceptor blocking agent with the BETA-ARM AGAT reagent kit. This method is based on the inhibition of erythrocyte hemolysis with beta-blocker use. Human erythrocytes undergo hemolysis, the degree of which is determined by the

Table 1

**Clinical and anamnestic characteristics of patients after MI,
depending on clinical course of heart failure**

Parameter	Group 1 (progressive heart failure) n=37	Group 2 (progressive heart failure) n=13	p
Age, Me (Q25;Q75), years	59,0 (48,5;63,5)	70,0 (49,0;78,0)	0,093
Male/Female, n (%)	29 (78,4)/8 (21,6)	11 (84,6)/2 (15,4)	0,99
CSS score, Me (Q25;Q75), points	2,0 (2,0;4,0)	5,0 (3,0;7,0)	0,006
EQ-5D-3L, Me (Q25;Q75) score, points	3,0 (2,0;4,0)	4,0 (3,0;5,0)	0,134
History of smoking, n (%)	9 (24,3)	3 (23,1)	0,99
Characteristics of MI:			
Q-wave MI, n (%)	28 (75,6)	8 (61,5)	0,462
STEMI, n (%)	34 (91,9)	10 (76,9)	0,257
Complications, n (%)	25 (67,6)	6 (46,2)	0,171
Coronary angiography data at the time of MI:			
Single-vessel stenosis \geq 50%, n (%)	3 (8,1)	4 (30,8)	0,065
Multi-vessel stenosis \geq 50% (two or more CA), n (%)	29 (78,4)	7 (53,8)	0,149
Echocardiographic data at the time MI:			
LVEF, Me (Q25; Q75), %	58,0 (51,3;63,0)	56,0 (44,5;61,5)	0,256
LVMI, Me (Q25; Q75), mm	98,0 (89,8;115,0)	112,5 (99,8;119,5)	0,043
End-systolic volume, Me (Q25; Q75), ml	45,0 (35,0;60,8)	53,0 (43,5;81,0)	0,123
End-diastolic volume, Me (Q25; Q75), ml	110,5 (87,5;125,0)	110,0 (105,0;161,5)	0,230
E/A, Me (Q25; Q75), CU	0,84 (0,74;1,19)	0,81 (0,71;1,26)	0,658
Background disease:			
Hypertension, n (%)	31 (83,8)	11 (84,6)	0,99
Obesity, n (%)	9 (24,3)	6 (46,2)	0,140
Type 2 diabetes, n (%)	5 (13,5)	1 (7,7)	0,99

Abbreviations: CA — coronary artery, CSS — clinical state scale, LVEF — left ventricular ejection fraction, LVMI — left ventricular mass index, Me (Q25; Q75) — median and interquartile range, MI — myocardial infarction.

optical density of the supernatant. The beta-blocker solution is added to the experimental sample, where it binds to the beta-receptors of the cell membrane and inhibits hemolysis. The optical density of the supernatant in the experimental sample is expressed as a percentage of the optical density in the control sample. Percent units are taken as conventional units (CU) of β -AWP. Normal ranges of β -AWP were considered from 2 to 20, which were proposed by the authors of this method (R.I. Stryuk and I.G. Dlusskaya (2003)). β -AWP >20 CU indicated reduced adrenergic reactivity, reflecting a decrease in the number of adrenergic receptors on the erythrocyte membrane.

Endpoint analysis (progression of HF) was performed after follow-up of 6 months. HF progression was recorded in cases of an increase in HF functional class.

This study was performed in accordance with the Helsinki declaration and Good Clinical Practice

standards. The medical ethics committee of Tomsk National Research Medical Center (Cardiology Research Institute) approved this study. All participants gave written informed consent.

Statistical processing was carried out using Statistica 10 and SPSS 20.0 (demo version) software. Qualitative characters are presented as absolute and relative values of n (%). Nominal data analysis was performed using the Pearson's chi-squared test and the two-sided Fisher's exact test if the expected character value in at least one cell of the contingency table was <5 . Analysis of quantitative data for distribution normality was carried out using the Shapiro-Wilk test. Quantitative characters corresponding to the normal distribution are presented as mean value and standard deviation ($M \pm SD$). Student's t-test was used in the case of a normal distribution and the equality of variances. Quantitative data that do not correspond to the normal distribution are presented

Table 2

Analysis of therapy in groups at the time of inclusion in the study

Parameter	Group 1 (stable heart failure) n=37	Group 2 (progressive heart failure) n=13	p
β-blockers, n (%)	32 (86,5)	11 (84,6)	0,99
ACE inhibitors, n (%)	20 (54,1)	11 (84,6)	0,095
ARB, n (%)	5 (13,5)	1 (7,7)	0,99
Diuretics, n (%)	6 (16,2)	5 (38,5)	0,09
MRA, n (%)	3 (8,1)	2 (15,4)	0,595

Abbreviations: ACE inhibitors — angiotensin-converting enzyme inhibitors, ARB — angiotensin II receptor blockers, MRA — mineralocorticoid receptor antagonists.

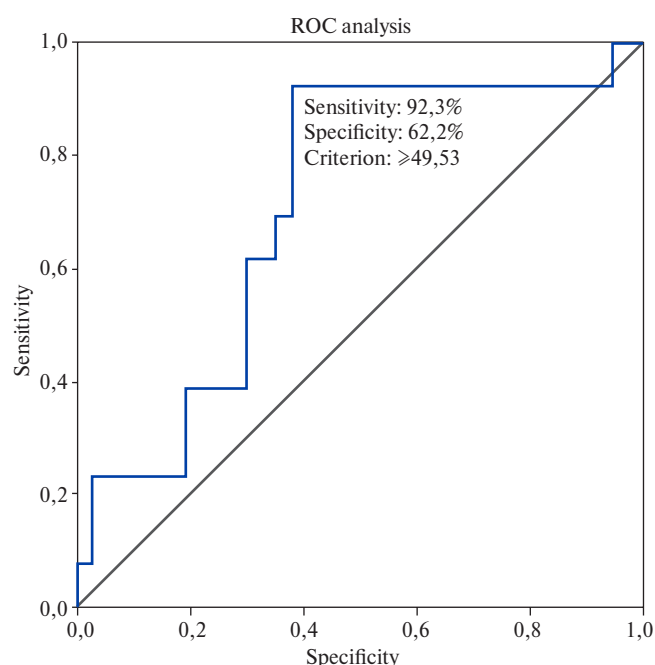


Figure 1. Sensitivity and specificity of β-AWP levels in stratification of the progression risk for heart failure in patients after MI (ROC analysis).

as the median and interquartile range (Me (Q25;75)). To compare the quantitative characters in two independent samples with non-normal distribution, the Mann-Whitney U-test was used. ROC analysis with the construction of a characteristic curve and the estimation of area under the curve (AUC) and odds ratio (OR) were performed to determine and characterize the associations between the studied factors. Differences were considered significant at $p < 0,05$.

Results

According to inclusion criteria, all patients after MI who were included in the study had HF. Distribution of patients depending on class was as follows:

class I — 23 patients (46%), class II — 19 patients (38%), class III — 8 patients (16%).

Analysis of MI revealed that the vast majority of patients were diagnosed with Q-wave MI (72%, $n=36$) and ST-segment elevation MI (88%, $n=44$). In general, index MI was characterized by a typical clinical picture (94%, $n=47$). Complications occurred in 62% of cases ($n=31$). Moreover, almost every second patient (42%, $n=21$) at the time of MI had a history of CAD, the duration of which in 24% of cases ($n=12$) was >5 years.

During the 6-month follow-up, HF progression was diagnosed in 26% of patients ($n=13$). All patients were divided into 2 groups, depending on the presence/absence of HF progression after MI. Group 1 included 37 patients with stable HF; group 2 — 13 patients with HF progression after MI (Table 1).

According to the CSS, the clinical status of group 2 patients was more severe: the score was 5 (3,0;7,0), which was 2,5 times higher than in group 1 (2 (2,0;4,0); $p=0,006$). However, the level of quality of life did not significantly differ in the studied groups ($p=0,1$). The clinical picture of MI, complications of acute MI, severity of coronary atherosclerosis, and the background diseases did not significantly differ between groups with stable and progressive HF ($p > 0,05$). Parameters of left ventricle (LV) structural and functional state also did not differ significantly between groups. However, according to echocardiography, LV mass index was slightly higher in group 2 patients ($p=0,043$), while LV hypertrophy was comparable.

At the time of inclusion in the study, there were no significant differences in taking certain groups of drugs in the studied groups. So, the main classes of drugs recommended for the HF treatment, such as β-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and mineralocorticoid receptor antagonists were prescribed equally often in both groups. The need for diuretic therapy was slightly higher in the group of patients with pro-

gressive HF (16,2% vs 38,5%). However, due to the small number of patients, these differences were not significant ($p=0,09$) (Table 2).

Analysis of beta-adrenergic reactivity at the inclusion time showed that patients of group 2 had higher β -AWP compared with group 1 (58,8 (50,9;78,0) CU vs 46,8 (38,0; 66,3) CU, respectively; $p=0,025$). At the same time, in both studied groups, the β -AWP values significantly exceeded standard rate of 20 CU.

To identify relationships between the beta-adrenergic reactivity levels and the probability of HF progression after MI, and to assess the possibility of using the β -AWP for stratifying the risk of HF progression in patients after MI, an ROC analysis was performed with AUC, which was 0,71 at $p=0,025$ (95% confidence interval (CI) 0,55-0,87). When analyzing the ROC curve, it was found that β -AWP $\geq 49,53$ CU can be considered as a marker of HF progression in patients after MI (sensitivity 92,3%, specificity 62,2%) (Figure 1).

We also found that β -AWP $\geq 49,53$ CU were observed in 37,8% of patients ($n=14$) in the group with a stable HF, while similar values of β -AWP in patients with progressive HF were diagnosed much more often — in 76,9% of patients ($n=10$) Odds ratio calculation showed that the level of β -AWP $\geq 49,53$ CU associated with a more than five-fold increase of HF progression risk in patients after MI (OR 5,48; 95% CI 1,28-23,37; $p=0,024$).

Discussion

The general position that catecholamine increase is accompanied by desensitization of membrane adrenergic receptors is confirmed by the numerous clinical and experimental studies. There is little number of studies on the functional state of SAS in HF patients, but the results of these studies reflect a significant excess of the average beta-adrenergic reactivity in HF patients compared with patients

without HF, as well as an increase in patients with more severe HF [4, 12, 14]. In earlier studies, the authors conclude that beta-adrenergic reactivity of cell membranes may be diagnostically valuable in assessing HF severity and, together with other clinical data, be a criterion for an individual reaction of organism in adaptation process during SAS activation [15]. All this indirectly confirm the theory that the adrenergic reactivity of erythrocytes to a certain extent reflects the general adrenergic reactivity of the organism and can be extrapolated to it [11].

Our study also confirms the hypothesis that the β -AWP value is significantly associated with the clinical course of HF in patients after MI. We showed that in patients with progressive HF after MI, a decrease in adrenergic reactivity (β -AWP increase) is characteristic, which is consistent with the results of previous studies in patients without a history of MI [14, 15]. In this study, β -AWP $\geq 49,53$ CU was first established as a marker of HF progression in patients after MI.

Conclusion

In patients with HF and MI history, there is a decrease in the adrenergic reactivity of erythrocyte cell membrane, which is reflected by an increase of β -ARM above normal range of 20 CU. At the same time, β -ARM in patients with HF progression compared with patients without it is significantly increased.

Established cut-off point of β -ARM ($\geq 49,53$ CU) allows predicting the HF progression with high sensitivity and specificity (92,3 and 62,2, respectively). β -AWP $\geq 49,53$ CU associated with a more than five-fold increase of HF progression risk in patients after MI (OR 5,48; 95% CI 1,28-23,37; $p=0,024$).

Relationships and Activities: not.

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