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Comparative analysis of adrenergic reactivity of erythrocytes in patients with myocardial infarction depending on the severity of coronary obstruction

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Aim. To study the parameters of beta-adrenergic reactivity of membrane (β-ARM) of erythrocytes in patients with myocardial infarction with nonobstructive coronary arteries (MINOCA) and single-vessel CAD.

Material and methods. The study included 40 patients with MI (experimental group — 19 patients; control group — 21 patients). Three patients (15,7%) with diagnosed acute myocarditis were excluded from the analysis. Levels of β-ARM were determined upon admission, on the 2nd, 4th and 7th day after MI. The normal range of β-ARM were <20 CU.

Results. In a significant proportion of patients, β-ARM values were two times higher than normal values. The median β-ARM in the experimental group at admission was 41,7 (29,0; 61,5) CU, on the day 1 — 48,6 (38,5; 57,3) CU, day 4 — 49,4 (39,0; 63,3) CU, day 7 — 53,5 (35,2; 67,7) CU. In the control group, the median β-ARM at admission was 52,5 (25,4; 64,5) CU, day 1 — 51,6 (28,3; 56,9) CU, day 4 — 48,5 (34,9; 61,2) CU, day 7 — 45,1 (32,2; 68,9) CU. Static analysis of β-ARM at all follow-up periods did not show differences between the groups by median level (p>0,05). The curves of β-ARM median changes show its multidirectional dynamics in the studied groups. During the hospitalization, in the group of patients with MINOCA there was a downward trend in β-ARM. In the control group, there was a tendency to increase of β-ARM. A statistically significant correlation of β-ARM with the ejection fraction (r=0,83, p=0,0007) and a moderate correlation between the β-ARM level on the 4th day and GRACE risk (r=0,55, p=0,03) in patients of the control group were revealed.

Conclusion. β-ARM values in patients with MINOCA were doubled, and this increase was comparable to levels in patients with obstructive CAD. During the hospitalization, the β-ARM levels did not significantly change, despite the use of beta-blockers.

Key words: nonobstructive coronary artery atherosclerosis, myocardial infarction with nonobstructive coronary arteries, beta-adrenergic reactivity of erythrocytes.

Relationships and Activities: The study was carried out as a part of the theme of fundamental scientific research № AAAA-A15-115123110026-3.

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Myocardial infarction (MI) with non-obstructive coronary arteries (MINOCA) is important and common clinical problem, which became clear after the introduction of invasive coronary angiography (ICA) into routine practice. According to large registers of acute myocardial infarction (AMI), the incidence of MINOCA is 5-15% [1-3]. An increasing number of studies on MINOCA did not provide comprehensive answers about the mechanisms of ischemia and its consequences.

It is known that hyperactivation of the sympathoadrenal system (SAS) is considered as one of the key causes of impaired cardiovascular homeostasis in patients with AMI [4]. Pathological activation of SAS causes a quantitative and functional change in adrenergic receptors, contributing to desensitization of cell membranes to stress mediators and hormones [5]. This is accompanied by a significant decrease in beta-adrenergic receptor (β-AR) density on the membrane of cardiomyocytes. With a reduced amount of β-AR and the action of catecholamines, myocardial oxygen demand increases and against the background of microvascular changes, vasospasm, non-obstructive atherosclerosis, it can contribute to AMI, characteristic of patients with MINOCA [6, 7].

At present, there is no unified method for assessing the activity of SAS, therefore, both direct and indirect approaches are used. Direct methods include the determination of blood mediators and hormones of SAS. These approaches are highly specific and sensitive, but at the same time labor-intensive and expensive. Indirect methods are as follows: assessment of the activity of enzymes for catecholamine synthesis and deactivation, determining the levels of electrolytes in blood and cortisol in saliva, as well as other methods [8]. In addition, one of such indirect methods is the determination of β beta-adrenergic reactivity of membrane (β-ARM) of erythrocytes. It is based on the fact that by binding to β-AR on the erythrocyte membrane, adrenergic agonists and blockers can alter the activity of hyposmolar hemolysis of these cells [4]. This method was developed and implemented by R. I. Stryuk and I. G. Dlusskaya, who for the first time studying adrenergic reactivity in patients with hypertension (HTN), revealed a 2-fold increase in β-ARM compared to the normal values. The authors concluded that values of β-ARM >20 CU can be used as an objective quantitative criterion for the hyperadrenergic type of HTN, and the β-ARM method can be considered a systemic indicator of adrenergic reactivity of the body [4]. This indicator characterizes both the sensitivity of cell membranes to catecholamines and the functional state of adrenergic receptors, which can dynamically alter with changes in SAS.

Previously, it was shown that β-ARM values were significantly increased in the early period after infarction in patients with AMI with obstructive coronary arteries [9, 10]. However, there are no data on the changes of β-ARM during and after AMI. In addition, a comparative assessment of adrenergic receptors in patients with MINOCA and AMI with single-vessel coronary stenosis is of interest. The central hypothesis that was tested in this study was that higher adrenergic reactivity is one of the leading factors for MINOCA.

The aim was to study the parameters of beta-adrenergic reactivity of membrane (β-ARM) of erythrocytes in patients with myocardial infarction with nonobstructive coronary arteries (MINOCA) and single-vessel coronary artery disease (CAD).

Material and methods

The non-randomized, open-label, controlled study was performed (ClinicalTrials.gov Identifier: NCT03572023. Ethics committee of the Cardiology Research Institute of Tomsk National Research Medical Centre approved this study (№ 164 of 11.23.2017). All patients signed informed consent.

The experimental group included patients over 18 years old with acute coronary syndrome (ACS) and non-obstructive CAD (intact coronary arteries or stenosis <50%) according to ICA performed within 24 hours from ischemia onset, with high and intermediate risk categories on the GRACE Score. The control group included patients over 18 years old with ACS, high and intermediate risk categories on the GRACE Score and obstructive single-vessel CAD (stenosis ⩾75%) according to ICA performed within 24 hours from ischemia onset. Patients with history of coronary revascularization were excluded from the study.

B-ARM was determined by assessing the osmotic fragility of erythrocytes after β-AR blockade *in vitro* by selective β-blocker using the BETA-ARM reagent kit (AGAT, Russia). Analysis of β-ARM was performed upon admission, on the 2nd, 4th and 7th day after AMI. Normal values of β-ARM are in the range from 2,0 to 20,0 CU, which reflects the high osmotic fragility of erythrocytes. An increase in β-ARM is a result of desensitization of β-AR on erythrocyte membranes. A decrease in the number of binding sites on the erythrocyte membrane leads to an increase in hyposmolar hemolysis. In addition, β-ARM values exceed 20 CU.

Dynamic measurement of cardiac enzymes was carried out upon admission, on the 2nd, 4th and 7th day from the ischemia onset. Conventional echocardiography was performed on the 4th day using the VIVID E9 ultrasound system (GE Healthcare).

Statistical analysis was carried out using the STATISTICA 10 software package. Hypothesis of a normal distribution was tested using the Shapiro-

Table 1

Clinical and anamnestic characteristics of patients

	MINOCA	Single-vessel lesion	p-value
Number of patients, n %	19 (100)	21 (100)	
Men, n (%)	7 (33,3)	17 (80,9)	0,04
Age, Me (Q25; Q75)	66 (51;71)	60 (56;68)	0,22
Hypertension, n (%)	15 (78,9)	16 (76,1)	0,83
Dyslipidemia, n (%)	15 (78,9)	17 (80,9)	0,62
Obesity, n (%)	13 (68,4)	11 (52,3)	0,30
Heredity*	9 (47,3)	12 (57,1)	0,35
Smoking, n (%)	8 (42,1)	16 (76,1)	0,11
Type 2 diabetes, n (%)	-	4 (19,0)	0,18
GFR, ml/min/1,73 m^2, Me (Q25; Q75)	69 (54,0;83,0)	79 (65,0;89,0)	0,17
History of angina, n (%)	11 (57,8)	6 (28,5)	0,04
History of stroke, n (%)	1 (5,2)	2 (9,5)	0,60
Peripheral atherosclerosis, n (%)	5 (26,3)	7 (33,3)	0,62
Time of admission to the hospital, min, Me (Q25; Q75)	390 (143;900)	180 (98;240)	0,02
STEMI, n (%)	12 (63,1)	19 (90,4)	0,03
GRACE score (Q25; Q75)	2,5 (2,0;9,0)	2,3 (2,0;5,0)	0,42
Prehospital TLT, made/effective	4 (21,0)/3 (15,7)	11 (52,3)/7 (33,3)	0,002

Note: * — positive family history for cardiovascular disease.
Abbreviations: GFR — glomerular filtration rate, TLT — thrombolytic therapy, MINOCA — myocardial infarction with non-obstructive coronary arteries.

Wilk test. Quantitative traits are presented as medians (Me) and quartiles (Q25; Q75). Qualitative traits are presented as n (%) (n — absolute number; % — relative percentage value). Nominal data were analyzed using the Pearson's chi-squared test and the Fisher's two-tailed exact test (expected frequencies <5). Due to the fact that the studied values were not normally distributed, the nonparametric Mann-Whitney U-test was used to assess the differences in the independent samples. To assess the significance of differences in the dependent samples, the nonparametric Friedman's test was used. To evaluate the correlation between the variables, the nonparametric Spearman's test was used. Multiple regression and logistic analyzes were performed. Differences were considered significant at $p<0,05$.

Results

The study included 40 patients with ST-segment elevation MI (STEMI) and non-STEMI (NSTEMI) (19 patients with MINOCA and 21 patients with MI with obstructive CAD). The median age of patients in the experimental and control groups was 66,0 (54; 71) and 60 (56; 68) years, respectively.

After a differential diagnosis, 3 (15,7%) patients with acute myocarditis diagnosed with magnetic resonance imaging and endomyocardial biopsies, were excluded from the study. Among them, one patient had a combination of myocarditis and pulmonary embolism.

The main clinical and anamnestic characteristics of patients of both groups are presented in Table 1. There were significant differences in gender, history of angina, diabetes, time of admission, and prehospital thrombolytic therapy (TLT).

All patients in the hospital received ACS therapy according to national guidelines: dual antiplatelet therapy (Cardiomagnyl; Clopidogrel/Ticagrelor), low-molecular-weight heparins, beta-blockers, statins, ACE inhibitors or sartans.

An analysis of examination data revealed that patients with MINOCA had significantly lower values of creatine phosphokinase (CPK), CPK-myocardial band (MB) on the 1^{st} and 4^{th} day and troponin I on the 1^{st}, 4^{th} and 7^{th} day compared with the control group. A higher level of C-reactive protein (CRP) was determined on the 1^{st} day in the experimental group. According to echocardiography, levels of end-diastolic and end-systolic volume, the left ventricular (LV) local contractility index were significantly higher in patients with MI with obstructive CAD. Examination data of the studied groups are presented in Table 2.

Table 2

Data of investigations

Parameter	MINOCA	Single-vessel lesion	p-value
CPK, u/l, at admission	309,5 (150,0;752)	350,5 (250,5;673)	0,46
CPK, u/l, 1 day	308,5 (140,5;614,0)	1178,5 (588,0;2409,0)	<0,001
CPK, u/l, 4 days	127 (60,0;353,0)	333,5 (139,5;653,5)	0,02
CPK, u/l, 7 days	85 (51,0;138,0)	97,0 (72,0;180,0)	0,24
CPK-MB, u/l, upon admission	38,5 (26,0;88,5)	45,9 (26,5;96)	0,56
CPK-MB, u/l, 1 day	31,0 (13,7;71,3)	173,5 (60,1;260,5)	<0,001
CPK-MB, u/l, 4 days	18,9 (12,0;23,6)	36,4 (19,5;59,5)	0,002
CPK-MB, u/l, 7 days	15,7 (12,0;19,0)	16,4 (11,8;22,1)	0,69
Troponin I, ng/ml, 1 day	0,65 (0,2;6,0)	4,9 (1,0;25,2)	0,01
Troponin I, ng/ml, 4 days	0,5 (0,08;1,9)	0,7 (0,5;4,4)	0,057
Troponin I, ng/ml, 7 days	0,09 (0,02;0,2)	0,4 (0,2;0,9)	<0,001
Cholesterol, mmol/L	4,5 (3,7;5,8)	4,5 (3,9;4,9)	0,43
TG, mmol/l	1,3 (0,9;2,5)	1,7 (1,1;2,0)	0,55
HDL, mmol/l	1,2 (0,9;1,4)	1,1 (1,1;1,3)	0,99
LDL, mmol/l	2,7 (2,1;3,4)	2,6 (2,2;2,8)	0,37
LDL/HDL	2,8 (1,6;3,0)	2,1 (1,8;2,3)	0,31
CRP, mg/l, 1 day	24,5 (3,8;42,0)	4,9 (4,1;27,3)	0,29
CRP, mg/l, 4 days	16,0 (4,8;20,0)	4,8 (3,9;17,9)	0,29
CRP, mg/l, 7 days	5,3 (3,4;10,0)	3,9 (3,5;12,4)	0,86
LV EDV, ml	95,0 (76,0;106,0)	101 (91,0;122,0)	0,11
LV ESV, ml	34,0 (28,0;45,0)	43,0 (35,0;53,0)	0,06
LCII, score	1,0 (1,0;1,2)	1,2 (1,2;1,5)	0,04
LVEF, %	60,0 (45,0;60,0)	56,0 (50,0;60,0)	0,51

Abbreviations: INLS — local contractility impairment index, EDV — end-diastolic volume, EDV — end-systolic volume, CPK — creatine phosphokinase, LV — left ventricle, HDL — high density lipoproteins, LDL — low density lipoproteins, CRP — C-reactive protein, TG — triglycerides, EF — ejection fraction, LCII — local contractility impairment index.

We found that in 85% of patients, the values of β-ARM were 2 times higher than normal values (Figure 1). Static analysis of β-ARM at all follow-up periods did not reveal significant differences between the groups by median level (p>0,05). The curves of changes in β-ARM medians during observation, presented in Figure 2, show its multidirectional dynamics. During hospitalization, patients with MINOCA had a downward trend in β-AWP, while patients with MI with obstructive CAD — an upward trend.

Correlation analysis revealed a moderate correlation between the level of β-ARM on the 4th day and the GRACE score (r=0,55, p=0,03) in patients with MI with obstructive CAD. In patients with MINOCA, a close correlation of β-ARM with the left ventricular ejection fraction (LVEF) was obtained (r=0,78, p=0,0007). The revealed correlation in patients with MINOCA was confirmed by multiple regression analysis, which were obtained by step-by-step inclusion of cardiovascular risk factors: age, sex, smoking, heredity; GRACE score, time of admission to the hospital; examination parameters: serum levels of CPK, CPK-MB, troponin I, CRP, end-diastolic volume, end-systolic volume, LV local contractility index and LVEF. It was revealed that the β-ARM level is correlated with age, heredity, risk (GRACE score), LVEF, and levels of myocardial necrosis markers:

Equation 1

– β-ARM level on the 4th day = -43,09 — 0,54 * GRACE score — 27,5 * heredity — 2,35 * CPK-MB, 4th day + 9,6 * troponin I, 4th day + 2,3 * LVEF.

Equation 2

– β-ARM level on the 7th day = -64,2 — 0,57 * age — 1,22 * CPK-MB, 7th day + 13,2 * troponin I, 7th day + 2,7 * LVEF.

Discussion

Before comparing β-ARM between groups, it was important to compare their clinical, history and exami-

nation characteristics. So, the median age of patients in the experimental group was 66 years, which is higher than in patients with MI with obstructive CAD. There were significantly lower number of men in the experimental group (43,7%), p=0,02. According to the analysis of risk factors, patients with MINOCA did not have diabetes, but statistically more often had a history of angina (62,5%), and for other risk factors, there were no differences, p>0,05. It was also found that 62,5% of patients with MINOCA had ST-segment elevation ACS, in contrast to the control group, where there were 90,4% of such patients (p=0,01), which is consistent with a higher frequency of prehospital TLT in the control group (p=0,007). However, according to the GRACE score, patients of both groups had a moderate cardiovascular risk, p=0,26 (Table 1). Attention is drawn to the low level of cardiac enzymes in patients with MINOCA, which indicates a small area of necrosis. These data are consistent with significant differences in the local contractility impairment index (p=0,007) (Table 2). We also revealed significant differences in CRP levels in patients with MINOCA on the first day after AMI (p=0,05), which may indicate a more aggressive atherosclerosis with plaque destabilization [11].

Thus, patients with MINOCA were older, later admitted to hospital, had a lower number of risk factors and a less pronounced increase in serum myocardial necrosis markers. This suggests a more favorable course of MI and less neurohormonal reactivity both during and after the hospitalization.

Based on the obtained data and theoretical assumptions, one would expect that the β-ARM data would be different in the studied groups. However, this hypothesis was not confirmed: β-ARM values were comparable, p>0,05. The medians of β-ARM in both groups were doubled (Figure 1), which generally corresponds to adrenergic hyperactivity and is characterized by an increase in SAS activity, and also indicates protective desensitization of β-AR in response to a change in their autonomic regulation due to AMI. Similar data were found in patients with AMI with obstructive atherosclerosis with different clinical course, however, β-ARM parameters were studied only on the 1st day from the index event [9]. The authors found the relationship of β-ARM with the clinical features of ST-segment elevation AMI. Thus, patients with complicated AMI had low β-ARM (β-ARM ⩽20 CU), which indicates a high risk of recurrent events due to the high sensitivity of β-AR to catecholamines. An increase in β-ARM (>20 CU), on the contrary, was a favorable prognostic factor. This indicates that the protective role of desensitization is activated, the amount of β-AR is reduced and this is manifested in various systems and organs, including the cardiac muscle [8]. At the same time,

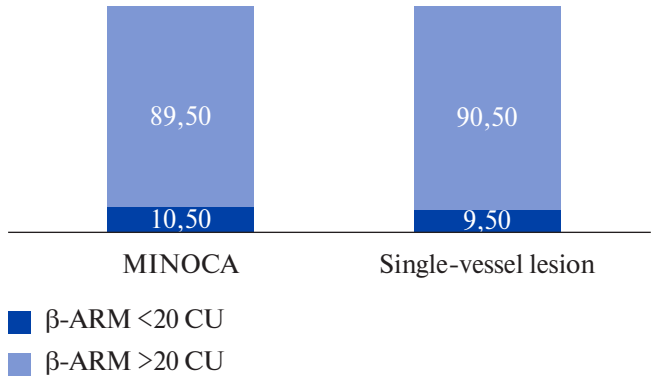

Figure 1. Patients with β-ARM <20 CU and >20 CU, %.

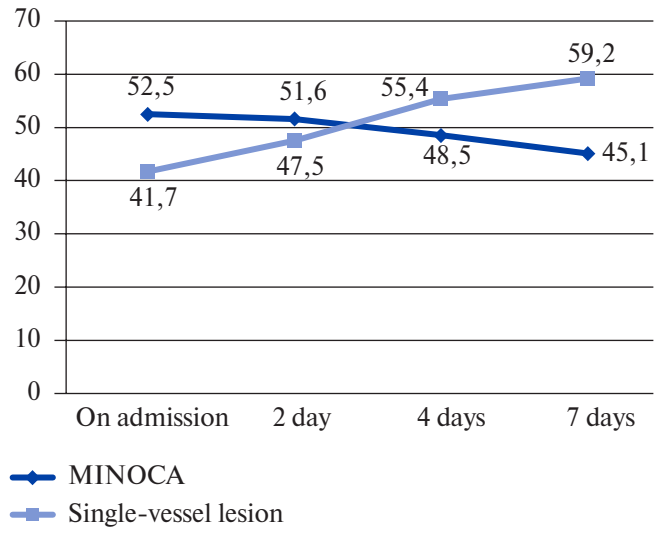

Figure 2. Changes of β-adrenergic reactivity of erythrocyte membranes in the studied groups.

the results of other studies confirm the hypothesis that prolonged stimulation with catecholamines during myocardial ischemia reduces the β-AR amount, ensures maintenance of the cardiac contractility, and is a criterion for a favorable prognosis in the period after MI [10, 12]. These data are consistent with the correlation of β-ARM with LVEF confirmed by multiple regression analysis, which is revealed in our study. The higher the β-ARM and LVEF, the more favorable prognosis in post-MI period in patients with MINOCA.

We revealed moderate correlation between the β-ARM level on the 4th day and the GRACE score in patients with MI with obstructive CAD. This can also be associated with the prognosis, since the GRACE score is an indicator of the prognosis after MI.

Changes of β-ARM medians in patients of both groups, presented in Figure 2, shows it multidirectionality, despite the fact that there were no signifi-

cant differences (p>0,05). In the group of patients with obstructive CAD, there was a trend towards an increase in β-ARM, while patients with MINOCA were more likely to have a β-ARM decrease. There is evidence in studies that long-term beta-blocker use leads to a decrease in β-ARM as a result of a decrease in β-AR desensitization [8, 13]. In the present study, 85% of patients took beta-blocker from the admission to the hospital, however, the upward trend of β-ARM was observed only in the control group, which is probably associated with the restoration of blood flow after myocardial revascularization, a decrease in stress mediators and hormones, partial desensitization of β-AR as a result of taking beta-blockers. Lack of significant changes in β-ARM parameters in patients with MINOCA is associated with continued activation of SAS and a decrease in the β-AR number, which in turn causes less binding of β-AR to β-blocker and is manifested by hyposmolar hemolysis of erythrocytes. Preserved hypersympathicotonia is probably associated with other pathogenetic mechanisms affecting this indicator. To answer questions about how long hypersympathicotonia preserves and how it is associated with a long-term prognosis, whether beta-blocker in patients with MINOCA reduces the effect of catecholamines, further large studies with a long-tern follow-up period is necessary.

Conclusion

During hospitalization, β-ARM values in patients with MINOCA were doubled, and this increase was comparable to levels in patients with obstructive CAD. Within the follow-up period, the β-ARM levels did not significantly change, despite the use of beta-blockers, which indicates the continued long-term desensitization of adrenergic receptors under the action of catecholamines.

Relationships and Activities. The study was carried out as a part of the theme of fundamental scientific research № AAAA-A15-115123110026-3.

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