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МОЛЕКУЛЫ АДГЕЗИИ В ОЦЕНКЕ ГОДОВОГО ПРОГНОЗА У МОЛОДЫХ БОЛЬНЫХ, ПЕРЕНЕСШИХ ОСТРЫЙ КОРОНАРНЫЙ СИНДРОМ

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Adhesion Molecules in Assessment of Annual Prognosis in Young Patients with Acute Coronary Syndrome

Резюме

Цель. Изучение места и роли адгезивных молекул (E-, L-, P-селектинов, молекул межклеточной и сосудистой адгезии 1 типа — ICAM-1, VCAM-1) в развитии неблагоприятного годичного прогноза у молодых пациентов с острым коронарным синдромом (ОКС). **Материал и методы.** В проспективное наблюдение продолжительностью 12 месяцев были включены 95 пациентов (90 мужчин, 5 женщин, средний возраст 41,00 [39,00-43,00] год) с ОКС, перенесших чрескожное коронарное вмешательство (ЧКВ). Оценивали конечные точки (первичная — смерть от сердечно-сосудистой патологии, вторичные комбинированные — нефатальные острый инфаркт миокарда и острое нарушение мозгового кровообращения, экстренные госпитализации ввиду сердечно-сосудистых причин — нестабильная стенокардия, аритмии, сердечная недостаточность). Методом иммуноферментного анализа в 1-е (до ЧКВ) и на 7-е сутки госпитализации (после ЧКВ) определяли концентрации в крови E-, L-, P-селектинов, молекулы межклеточной адгезии 1 типа (ICAM-1), сосудистой молекулы адгезии 1 типа (VCAM-1). **Результаты.** В течение 12 месяцев 22 (23,16 %) пациента имели оцениваемые конечные точки: смерть — у 2 (2,1 %) пациентов, нефатальный инфаркт миокарда — у 6 (6,32 %), госпитализация в связи с нестабильной стенокардией — у 14 (14,73 %). У пациентов с ОКС с неблагоприятным годовым прогнозом количество лейкоцитов, уровни P-селектина и ICAM-1 в 1-е сутки (до ЧКВ), L- и P-селектинов на 7-е сутки после ЧКВ были существенно выше, чем у пациентов с благоприятным годовым периодом. По данным многофакторного анализа, предикторами неблагоприятного годового прогноза у молодых пациентов с ОКС являлись уровни лейкоцитов (p=0,020) и ICAM-1 (p=0,010) в 1-е сутки (до ЧКВ); L-селектина — на 7-е сутки после ЧКВ (p=0,040). **Заключение.** У молодых пациентов с ОКС наиболее значимыми факторами, определяющими неблагоприятное течение острых форм ишемической болезни сердца в течение первых 12 месяцев, являются уровни лейкоцитов крови и ICAM-1 в 1-е сутки (до ЧКВ), L-селектина — на 7-е сутки после чрескожного коронарного вмешательства.

Ключевые слова: *острый коронарный синдром, молодой возраст, молекулы адгезии, прогноз*

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Abstract

Aim. To study the place and role of adhesive molecules (E-, L-, P-selectins, intercellular and vascular adhesion molecules of type 1 — ICAM-1, VCAM-1) in development of unfavorable one-year prognosis in young patients with acute coronary syndrome (ACS). **Material and methods.** A 12-month prospective follow-up included 95 patients (90 men, 5 women, mean age 41.00 [39.00-43.00] years) with ACS who underwent percutaneous coronary intervention (PCI). The endpoints were evaluated (primary — cardiovascular death, and combined secondary — nonfatal acute myocardial infarction and acute cerebrovascular accident, emergency hospitalization due to cardiovascular causes — unstable angina, arrhythmias, heart failure). Blood concentrations of E-, L-, P-selectins, ICAM-1, VCAM-1 were determined by enzyme immunoassay on the 1st (before PCI) and on the 7th day of hospitalization (after PCI). **Results.** During 12 months, 22 (23.16 %) patients had estimated endpoints: death in 2 (2.1 %) patients, nonfatal myocardial infarction in 6 (6.32 %), hospitalization due to unstable angina in 14 (14.73 %). In ACS patients with unfavorable annual prognosis, the levels of leukocytes, P-selectin and ICAM-1 on day 1 (before PCI), L- and P-selectins on day 7 (after PCI) were significantly higher than in patients with favorable annual period. According to multivariate analysis, predictors of unfavorable annual prognosis in young ACS patients were the levels of leukocytes (p=0.020) and ICAM-1 (p=0.010) on day 1 (before PCI) and L-selectin on day 7 after PCI (p=0.040). **Conclusion.** During the first 12 months in young ACS patients the most significant factors of unfavorable prognosis are the levels of blood leukocytes and ICAM-1 on day 1 (before PCI), and L-selectin on the 7th day after percutaneous coronary intervention.

Key words: *acute coronary syndrome, young age, adhesion molecules, prognosis*

Conflict of interests

Co-author of the article Yagoda A.V. is a member of the editorial board of the journal «The Russian Archives of Internal Medicine». The article passed the journal's peer review procedure. Yagoda A.V. was not involved in the decision to publish this article. The authors did not declare any other conflicts of interest

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Conformity with the principles of ethics

The study protocol was approved by the local ethics committee of the Stavropol State Medical University (Protocol No. 59 dated 11/17/2016). All patients signed informed consent.

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CI — confidence interval, ACS — acute coronary syndrome, OR — odds ratio, PCI — percutaneous coronary intervention, ICAM-1 — intercellular adhesion molecule, type 1, ROC — receiver operating characteristic, VCAM-1 — vascular cell adhesion molecule, type 1

Introduction

Wide use of advanced interventional therapies, efficient drugs and developed mapping of patients with acute coronary syndrome resulted in significant reduction in the rate of cardiovascular complications during the hospital period [1]. At the same time, patients surviving acute coronary syndrome are still at risk of cardiovascular events, especially during the first year after the index event [1, 2]. In this context, of interest is the so-called residual risk of cardiovascular complications, which is interpreted also based on parameters of non-specific inflammation [1, 3].

An inflammatory reaction in acute coronary syndrome patients presents with elevated levels of white blood cells, fibrinogen, C-reactive protein, cytokines, adhesion molecules and a number of other pro-inflammatory factors [4]. It is assumed that the inflammation intensity during the first day of acute coronary syndrome (ACS) is the determinant of the post-hospital clinical outcome [5]. At the same time, the study of prognostic capabilities using adhesion molecules, i.e. factors, which are induced as a response to inflammation, provided quite controversial results. For example, it has been established that higher circulating levels of intercellular adhesion molecule, type 1 (ICAM-1), vascular cell adhesion molecule, type 1 (VCAM-1), and P-selectin correlate with unfavourable clinical events within 12 months after the acute coronary process [5, 6, 7]. Other studies demonstrated opposite findings: lack of any association between high ICAM-1, E- and P-selectin levels with a risk of recurrent cardiovascular events [8, 9]. It can be assumed that these differences in study results are caused by abundance of mechanisms of ACS development, obstructive and non-obstructive coronary artery atherosclerosis or presence of intact coronary arteries, as well as differences in patient age, comorbidities, i.e. factors, which greatly affect biomarker concentration.

It is worth mentioning that patients, whose ischaemic heart disease manifested in the young age, differ from older age groups in the structure of the risk factors, clinical presentation and prognosis [10]. At the same time, cases of obstructive atherosclerotic damage to coronary arteries in young people are not uncommon [11]. The prognostic value of adhesion molecules, which play a significant role also in atherosclerotic damage through cell-cell interaction mediation between endothelium, monocytes, smooth muscle cells, platelets, in young patients remains unclear and is of tremendous clinical interest.

Therefore, it is advisable to measure the concentration of adhesion molecules in addition to routine inflammation markers — WBC, C-reactive protein, fibrinogen — in young patients with ACS in order to predict the risk of poor outcome, based on a one-year follow-up; one year is a period, when the majority of cardiovascular recurrences occur. A study of adhesion molecules in combination with other inflammation markers can provide a more accurate patient stratification depending on the risk of poor prognosis, which can have not only medical, but also socioeconomic significance.

The objective of this study was to study the place and value of adhesion molecules (E-, L-, P-selectins, ICAM-1, VCAM-1) in the development of poor year-long prognosis in young patients with acute coronary syndrome.

Materials and methods

The study included 100 patients (93 men, 7 women, mean age: 41.00 [39.00–43.00] years), hospitalised with ACS. Inclusion criteria: signed informed consent form; acute coronary syndrome within 24 hours before hospitalisation, which is caused by atherosclerotic events (type 1 myocardial infarction, unstable angina, classes IB and IIIB under the classification by E. Braunwald); male and female subjects aged 25 to 44 years old. Exclusion criteria: type 2–5 acute myocardial infarction; unstable angina, classes A and C, and unstable angina, class IIB under the classification by E. Braunwald; patients after cardiopulmonary resuscitation or electrical cardioversion on disease onset; acute and chronic inflammatory conditions (period exacerbation); clinically significant comorbidity (with hepatic, renal, cardiac, and respiratory insufficiency); autoimmune diseases and cancer. Withdrawal criteria: refusal to participate at any time during the study. The study protocol was approved by the Local Ethics Committee at the Stavropol State Medical University.

Table 1. *Initial characteristics of young patients with acute coronary syndrome*

Indicator	Patients n (%)
Men	93 (93 %)
Women	7 (7 %)
Acute coronary syndrome with ST segment elevation	43 (43 %)
Acute coronary syndrome without ST segment elevation	57 (57 %)
Myocardial infarction type 1 with ST segment elevation	43 (43 %)
Myocardial infarction type 1 without ST segment elevation	14 (14 %)
Unstable angina pectoris of class IIIB	43 (43 %)
Anamnesis of angina pectoris	45 (45 %)
Anamnesis of myocardial infarction, including with PCI	35 (35 %) 14 (14 %)
Burdened hereditary anamnesis	59 (59 %)
Smoking	71 (71 %)
Excess body weight	41 (41 %)
Obesity	39 (39 %)
Arterial hypertension	22 (22 %)

Comments: PCI — percutaneous coronary intervention.

Baseline patient characteristics are presented in Table 1.

Coronary angiography results show that single-vessel coronary disease is diagnosed in 50 (50 %) patients; two damaged coronary arteries — in 31 (31 %) patients, while three and more coronary arteries were damaged in 19 (19 %) patients. All patients underwent stent placement in their symptoms-dependent artery. Standard laboratory and instrumental tests were performed, including blood WBC, C-reactive protein and fibrinogen on day 1 (before and after PCI). All patients had optimal drug therapy (according to the current clinical guidelines): DAPT, statins, angiotensin-converting enzyme inhibitors/ angiotensin receptor antagonists, β-blockers.

On day 1 (before PCI) and day 7 of hospitalisation (after PCI), all patients underwent plasma measurements of L-, E-, P-selectin, serum ICAM-1, VCAM-1 levels using ELISA test kits (Cloud-Clone Corp., China).

Over the next 12 months after admission, the following endpoints were evaluated: primary (cardiovascular-related deaths) and secondary (non-fatal acute myocardial infarction and acute cerebrovascular accidents, emergency hospital admission for cardiovascular causes: unstable angina, arrhythmias, cardiac insufficiency), as well as compliance.

Statistical data analysis was performed using StatTech, v. 4.2.7 (Russia). Normality of distribution was

checked using и the Kolmogorov-Smirnov test. Quantitative variables were presented with the median value and interquartile range (Me [Q25; Q75]), or mean ± standard error of mean (M±m). Qualitative parameters are presented as absolute values and percent. Differences between groups were identified using non-parametric Mann-Whitney U test or Student t-test for normal data distribution. A multivariate analysis was performed using the multivariate logistic regression analysis. ROC-analysis was used to evaluate the accuracy of the regression model and individual biomarkers. The level threshold was set in the cut-off point using the highest Youden's index. Odds ratios (OR) with 95 % confidence interval (CI) were calculated. Differences were statistically significant at $p < 0.05$.

Results

The information on the prognosis one year after the index ACS was obtained from 95 (95 %) patients. Twenty-two (23.16 %) patients experienced adverse events (endpoints), which were recorded only during the post-hospital period. Over the follow-up period, two 2 (2.1 %) patients dies; six (6.32 %) developed non-fatal myocardial infarction, and 14 (14.73 %) were hospitalised for unstable angina. There were no cases of acute cerebrovascular accidents and/or arrhythmia requiring hospitalisation, cardiac insufficiency among the endpoints of the study.

Given a small amount of events in each endpoint, it was decided to introduce a combined endpoint, which would increase the statistical power of prognosis;

Table 2. *Some markers of inflammation in blood of patients depending on annual prognosis*

Indicator	Pick-up time	Unfavorable prognosis		p
		Yes (n=22)	No (n=73)	
White blood cells, $\times 10^9$/l	1 day (before PCI)	12,26 ±3,67	10,32 ±3,33	**0,021**
	1 day (after PCI)	9,55 [8,79; 10,46]	8,29 [6,90; 9,80]	0,071
C-reactive protein, mg/l	1 day (before PCI)	4,50 [3,12; 5,92]	5,60 [3,50; 11,58]	0,161
	1 day (after PCI)	6,30 [3,53; 5,57]	4,50 [3,75; 5,20]	0,292
Fibrinogen, g/l	1 day (before PCI)	4,04 [3,40; 9,11]	3,64 [3,18; 4,90]	0,562
	1 day (after PCI)	4,46 [3,71; 5,57]	4,50 [3,75; 5,20]	0,941
L-selectin, ng/ml	1 day (before PCI)	249,50 [122,00; 504,00]	257,00 [112,00; 404,00]	0,411
	7 day (after PCI)	556,00 [314,00; 891,25]	230,00 [104,00; 554,00]	**0,010**
P- selectin, ng/ml	1 day (before PCI)	406,00 [261,50; 703,62]	250,70 [136,70; 411,30]	**0,010**
	7 day (after PCI)	545,25 [285,75; 691,25]	206,20 [166,50; 311,70]	**0,010**
E- selectin, ng/ml	1 day (before PCI)	31,30 [19,47; 38,68]	29,10 [21,50; 43,50]	0,952
	7 day (after PCI)	39,00 ±18,65	31,27 ±15,45	0,058
ICAM-1, ng/ml	1 day (before PCI)	1664,20 [962,30; 2341,30]	864,00 [698,80; 1358,40]	**0,010**
	7 day (after PCI)	1426,20 [905,88; 1868,20]	1049,50 [817,20; 1358,40]	0,177
VCAM-1, ng/ml	1 day (before PCI)	530,00 [420,00; 595,52]	468,00 [395,00; 550,00]	0,215
	7 day (after PCI)	1192,55±326,92	1136,93±437,10	0,581

Comments: PCI — percutaneous coronary intervention, ICAM-1 — intercellular adhesion molecule 1, VCAM-1 — vascular cellular adhesion molecule 1. Data with normal distribution is presented as M±m, Me [Q25; Q75]

Table 3. Results of multivariate analysis by logistic regression

Indicator	Regression coefficient (B)	Exponent B	95 % CI	p
White blood cells on day 1 (before PCI)	0,205	1,228	1,036-1,455	**0,020**
ICAM-1 on day 1 (before PCI)	0,001	1,001	1,001-1,002	**0,010**
L-selectin on day 7 (after PCI)	0,002	1,002	1,001-1,003	**0,040**
Constant	-5,888	-	-	-

Comments: CI — confidence interval, PCI — percutaneous coronary intervention, ICAM-1 — intercellular adhesion molecule 1.

this combined endpoint included death, acute myocardial infarction, and unstable angina. Depending on the outcome over the one-year follow-up period, patients were divided into two groups: group 1 (n = 22) — with poor one-year prognosis, and group 2 (n = 73) — with favourable one-year period.

Of note, there were no significant differences in traditional cardiovascular risk factors, past medical history, clinical variant of index ACS, severity of coronary damage, and compliance ($p > 0.05$).

Comparison of inflammation parameters in the study groups demonstrated statistically significant differences in WBC, P-selectin and ICAM-1 levels on day 1, L- and P-selection on day 7 of the disease (Table 2). C-reactive protein, fibrinogen, E-selectin, and VCAM-1 during the acute stage of the disease did not have any impact on recurrent cardiovascular events.

The identified statistically significant parameters observed in patients with poor one-year outcome were included into a multifactorial analysis. Logistic regression demonstrated an independent contribution to one-year poor prognosis from three out of five factors: WBC and ICAM-1 measured on day 1, and L-selectin, measured on day 7. P-selectin levels, both on day 1 (before PCI) and day 7 after PCI, did not have independent effects on poor one-year prognosis. Characteristics of each factor included into the model are presented in Table 3. Based on regression coefficients, WBC and ICAM-1 measured on day 1, and L-selectin, measured on day 7, had direct correlation with the probability of poor one-year prognosis.

At the next stage of the study, a model was generated which combined all independent prognostic factors:

$$P = \frac{1}{1 + 2.72^{-(-5.888 + 0.205X1 + 0.001X2 + 0.002X3)}},$$

Where P is the probability of identifying one-year poor prognosis; 2.72 is the base of the natural logarithm; –5.888 is the mathematical constant; 0.205, 0.001 and 0.002 are respective coefficients; X_1 is blood WBC before PCI (10^9/L); X_2 is pre-PCI ICAM-1 level (ng/mL); X_3 is L-selectin concentration on day 7 after PCI (ng/mL). The resulting regression model is statistically significant ($p < 0.001$).

We have conducted ROC analysis, which allowed us to find the limit of log function P. The resulting curve is presented in Figure 1. The area under ROC curve is 0.76±0.05 (95 % CI 0.65–0.86), indicating good quality of the model.

The cut-off limit threshold, which corresponded to the highest Youden's index, was 0.35, with sensitivity and specificity of 77.3 % and 72.6 %, respectively. Therefore, if the calculated P vale falls within the range from 0.35 to 1, then the poor one-year prognosis is highly likely. If P value is 0 to 0.35, then the probability of the poor one-year prognosis is low.

ROC analysis was used to identify the diagnostic accuracy of some biomarkers — WBC, ICAM-1 levels on day 1 (before PCI) and L-selectin concentration on day 7 after PCI (Fig. 2). For WBC, the identified limit of $10.6x10^9$/L possessed satisfactory sensitivity (77.3 %), but insufficient specificity (57.5 %). For ICAM-1 and L-selectin, the limits associated with prognosis were 1,240.0 ng/mL (sensitivity 63.6 %, specificity 79.5 %)

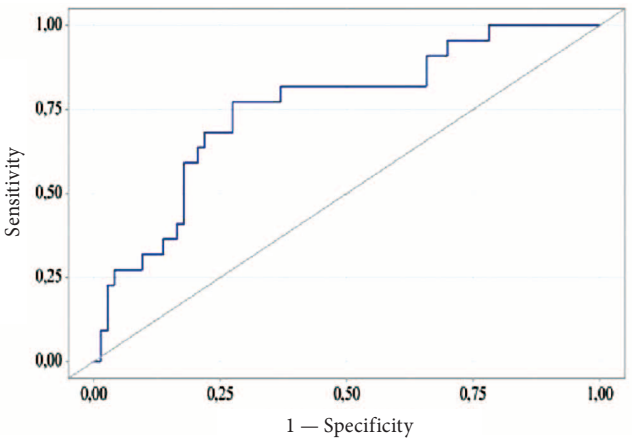

Figure 1. *ROC curve for model of predicting an unfavorable annual outcome in young patients with acute coronary syndrome*

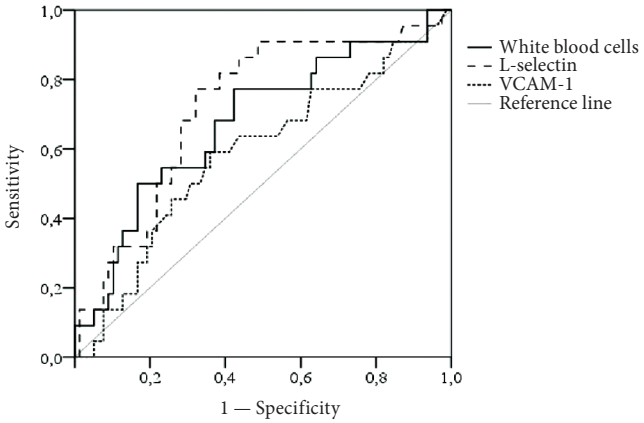

Figure 2. *ROC curves of diagnostic accuracy of inflammatory markers*
Comments: VCAM-1 — vascular cellular adhesion molecule 1.

and 314.00 ng/mL (sensitivity 77.3 %, specificity 65.8 %), respectively. Thus, unlike WBS values, adhesion molecules are more specific in indicating chronic inflammatory condition typical for atherosclerosis.

It has been shown that, in young patients with ACS, pre-PCI WBC of over $10.6x10^9$/L is associated with c 4–5-fold increase in the risk of recurrent cardiovascular conditions during the next year (OR 4.64; 95 % CI 1.55–13.84), pre-PCI ICAM-1 of over 1,240.0 ng/mL — with 5–6-fold increase in the risk (OR 5.82; 95 % CI 2.08–16.25), and L-selecting of more than 314 ng/mL (on day 7 after PCI) — with 6–7-fold increase in the risk (OR 6.8; 95 % CI 2.23–20.48).

Discussion

A high patient response rate (95 %) made it possible to conduct a correct analysis of factors affecting one-year prognosis. The absence of adverse cardiovascular events during hospitalisation is probably a result of the use of endovascular methods: revascularisation was performed in all patients included in the study. During the post-hospital period (one-year follow-up), the combined endpoint, including cardiovascular-related death, myocardial infarction and emergency hospitalisation with unstable angina, was recorded in 22 (23.16 %) patients, corresponding to the literature data (14.6–24.8 %) [1, 12, 13]. It is obvious that the residual risk of cardiovascular events during the first year after ACS remains high, and even advanced optimal therapies cannot compensate it.

Poor one-year prognosis in young patients with ACS was associated with high levels of WBC, P-selection and ICAM-1 on day 1, and L- and P-selectins on day 7. It is likely that higher concentrations of the mentioned adhesion molecules indicate the intensity and persistence of inflammation, causing more marked endothelial dysfunction and resulting atherosclerotic process destabilisation with elevated blood-clotting.

Absence in this study of prognostic significance of other inflammation markers, particularly of C-reactive protein, fibrinogen, E-selectin, VCAM-1 may be a result of their measurement timing. It is reported that C-reactive protein, used as a marker of poor prognosis, should be measured later, before discharge or a month after the index event [14].

The multivariate analysis demonstrated that independent predictors of poor one-year prognosis in young post-ACS patients are WBC and ICAM-1 levels on day 1 (before PCI), L-selectin — on post-PCI day 7. According to earlier information, WBC count was associated not only with larger myocardial infarction area and complications, but also with reduced therapy efficacy and patient survival rates during three to six months after infarction [4]. At the same time, it was demonstrated that in old patients with ACS, elevated blood WBC levels were not a risk factor for recurrent atherothrombotic events during one-year post-hospital follow-up [15]. Accordingly, it is worth noting that in old patients with ACS, low L-selectin levels have prognostic significance, indicating functional depletion of WBC [6]. In this study, young patients with ACS had high L-selectin levels on day 7 after coronary angioplasty as a marker of poor one-year prognosis. It is obvious that poor ACS prognosis in young patients is characterised with elevated WBC levels and their long-lasting activity, ensuring L-selectin generation. The prognostic potential of high ICAM-1 levels is probably independent of age: lower ICAM-1 levels found in this study on day 1 as an independent predictor of poor outcome in young patients with ACS was also a predictor of recurrent ACS in old patients, including after stent placement [13].

Measuring WBC, ICAM-1 on day 1 and L-selectin on day 7 makes it possible to clarify the risk of recurrent cardiovascular conditions and to minimise risk underestimation in young patients. Not also specific markers, but also timeline for their measurement, are crucial for patients with ACS.

These results can be used in clinical settings to improve classification of risks of poor one-year outcome in post-ACS young patients; it can be later used as novel targets in the therapy of acute IHD.

Study limitations A number of exclusion criteria used, particularly acute inflammatory conditions and recurrences of chronic conditions, clinically significant comorbidity (with hepatic, renal, cardiac, and respiratory

insufficiency), autoimmune diseases and cancer, could have independently contributed to the model of one-year prognosis in young patients with ACS. The resulting data can be extrapolated for young patients with ACS, provided it transforms to type 1 myocardial infarction and/or unstable angina, class IIIB (under the classification by E. Braunwald).

Conclusions

Poor course of ischaemic heart disease during the first 12 months after the PCI with stent placement was reported in 22 (23 %) young patients with ACS. In patients with poor prognosis, WBC, P-selectin and ICAM-1 levels on day 1 (before PCI) and L- and P-selectin levels on day 7 after PCI were significantly higher than in patients with favourable one-year prognosis. The multivariate analysis demonstrated that predictors of poor one-year prognosis in young patients with ACS are WBC ($p = 0.020$) and ICAM-1 ($p = 0.010$) levels on day 1 (before PCI) and L-selectin concentration on day 7 after PCI ($p = 0.040$). Limit thresholds for WBC and ICAM-1 on day 1 (before PCI) and L-selectin on day 7 were established, which are associated with 4–7-fold increase in the risk of recurrent cardiovascular events. These data allow recommending inclusion of adhesion molecules to an additional examination of young patients with ACS, making it possible to identify the risk group of poor course of the disease during the first year after PCI with stent placement.

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All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

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Eremenko A.M.: concept and design development, data collection, analysis and interpretation, justification and writing of the manuscript, final approval for publication, the author is responsible for all aspects of the work

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