DOI 10.26724/2079-8334-2022-3-81-148-152
UDC 616.13-002:616.12-005.4-08

V.O. Romanova, N.V. Kuzminova, T.V. Marchak, S.E. Lozinsky, I.I. Knyazkova[1], O.M. Kulchytska, Yu.L. Shkarivsky
Vinnytsia National Pirogov Memorial Medical University, Vinnytsia
[1]Kharkiv National Medical University, Kharkiv

PROGNOSTIC SIGNIFICANCE OF MARKERS OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH CORONARY HEART DISEASE

e-mail: kuzminova5517@gmail.com

The data of 135 patients with an uncomplicated course of the disease and 27 with an unfavorable outcome were analyzed for assessment of the prognostic significance of markers of endothelial dysfunction as predictors of an unfavorable prognosis in patients with coronary artery disease. A prospective 2-year study showed that unfavorable cardiovascular events were observed significantly more often in the group of patients with coronary artery disease with an initial elevated level of biomarkers of endothelial dysfunction compared to individuals whose level of these biomarkers was within the reference values. The presence of myocardial infarction at the start of observation, decreased left ventricular ejection fraction <50 % and increased levels of endothelial dysfunction markers: pregnancy-associated plasma protein A, soluble vascular cell-adhesion molecules and, to a lesser extent, endothelin-1 – have a significant predictive value in terms of the probability of adverse cardiovascular events.

Key words: endothelial dysfunction, coronary artery disease, patients' prognosis, pregnancy-associated plasma protein A, soluble vascular cell-adhesion molecules, endothelin-1.

В.О. Романова, Н.В. Кузьмінова, Т.В. Марчак, С.Е. Лозинський, І.І. Князькова, О.М. Кульчицька, Ю.Л. Шкарівський

ПРОГНОСТИЧНА ЗНАЧИМІСТЬ МАРКЕРІВ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКЦІЇ У ХВОРИХ НА ІШЕМІЧНУ ХВОРОБУ СЕРЦЯ

З метою оцінки прогностичної значимості маркерів ендотеліальної дисфункції в якості предикторів несприятливого прогнозу у хворих на ІХС були проаналізовані дані 135 пацієнтів з неускладненим перебігом захворювання і 27 – з несприятливим результатом. Проведене 2-річне проспективне дослідження показало, що несприятливі серцево-судинні події достовірно частіше виникали у хворих на ІХС з вихідним підвищеним рівнем біомаркерів ендотеліальної дисфункції порівняно з особами, у яких рівень цих біомаркерів був у межах референтних величин. Значною передбачуваною цінністю в плані ймовірності настання несприятливих подій володіли наявність інфаркту міокарда на початку дослідження, зниження фракції викиду лівого шлуночка <50 % та підвищення рівнів показників ендотеліальної дисфункції: асоційованого з вагітністю протеїну плазми А, розчинних судинних молекул адгезії і меншою мірою ендотеліну-1.

Ключові слова: ендотеліальна дисфункція, ішемічна хвороба серця, прогноз пацієнтів, асоційований з вагітністю протеїн плазми А, розчинні судинні молекули адгезії, ендотелін-1.

The study is a fragment of the research project "Metabolic risk factors, cardiovascular remodelling and functional state of kidneys in patients with cardiovascular pathology. Possibilities of pharmacological correction", state registration No. 0119U101849

Despite significant scientific achievements in clinical medicine, cardiovascular (CV) diseases, and primarily coronary artery disease (CAD), still dominate the structure of morbidity and mortality in various countries of the world, including Ukraine [2, 14]. The most common cause of death in patients with CAD is acute atherothrombotic events associated with impairment of the integrity of atherosclerotic plaque and subsequent thrombus formation. Therefore, searching for markers of increased atherothrombotic risk for early prediction of irreversible myocardium changes is a pressing task because it is impossible to predict these events at the moment [5, 6].

The primary pathophysiological mechanism of CAD is an imbalance between the myocardial oxygen demand and oxygen supply due to coronary atherosclerosis and, less often, coronary spasms and dysfunction of the heart's microvessels. Atherosclerosis, according to modern concepts, is a disease that affects arteries of elastic and muscular type and is characterized by latent inflammation of vessels, endothelial dysfunction, deposition of lipids, calcium, and cellular detritus in the intima with subsequent plaque formation, remodeling of vessels, their acute and/or chronic obstruction, disruption of laminar blood flow and reduction of oxygen supply to the affected organs.

Endothelial dysfunction is one of the essential chains in the pathogenesis of atherosclerosis [4]. It assumes an imbalance between the production of vasodilating, angioprotective, antiproliferative factors on the one hand and vasoconstrictor, prothrombotic, and proliferative factors on the other. Endothelial

dysfunction, a universal defect of the vascular wall, is an essential initial link of the cardiovascular continuum, determining the course and prognosis of cardiovascular diseases. Today, the endothelium of the intima of vessels is considered a powerful paracrine organ. It performs barrier, secretory, hemostatic, and vasoregulating functions and plays a vital role in inflammation and vascular wall remodeling.

The level of endothelin-1 (ET-1), whose vasoconstrictor potential is ten times higher than angiotensin II, is considered a potential marker of the regulatory function of the endothelium. Since the content of ET-1 in unstable atherosclerotic plaques is much higher than in stable ones [7, 15], it can be thought that it affects its destabilization. However, the importance of ET-1 in the formation and progression of the atherosclerotic process remains poorly understood.

The prothrombotic potential of the vascular endothelium is associated with the adhesion of monocytes and platelets to activated endothelial cells due to the overexpression of vascular adhesion molecules (sVCAM) on their surface. Although endothelial cells do not express adhesion molecules under physiological conditions, there is evidence that in practically healthy people, increased content of sVCAM is associated with a high risk of developing atherothrombotic complications of CAD [1]. Therefore, whether sVCAM-1 predicts future cardiac events in initially healthy individuals or only in individuals with existing coronary atherosclerosis remains debatable.

In 2001, in blood and in damaged atherosclerotic plaques in 8 patients with coronary heart disease who died suddenly, A. Bayes-Genis et al. discovered a zinc-containing matrix metalloproteinase – pregnancy-associated plasma protein A (PAPP-A) [10], which is synthesized in endothelial cells in response to damage and contributes to plaque instability, causing degradation of the extracellular matrix of the fibrous capsule.

The purpose of the study was to assess the prognostic significance of markers of endothelial dysfunction as predictors of an unfavorable prognosis in patients with coronary artery disease.

Materials and methods. The study included 173 patients with coronary heart disease (124 men and 49 women (mean age – 57.24±5.12 years)). They were initially treated as an inpatient in the cardiology department of the Vinnytsia Regional Clinical Pirogov Memorial Hospital and the department for patients with myocardial infarction of the "Vinnytsia Regional Clinical Medical and Diagnostic Center for Cardiovascular Pathology". Then they were subsequently observed on an outpatient basis for 24 months. Coronary heart disease was combined with hypertension in 82 patients (47.4 %).

After the examination, the patients were divided into 2 main clinical groups - 92 patients with stable CAD (45 II and 47 III functional classes, respectively) and 81 patients who were admitted to the hospital with the acute coronary syndrome (43 patients were diagnosed with unstable (progressive) angina, at 38 – acute myocardial infarction (MI)).

The diagnosis of stable coronary heart disease and the acute coronary syndrome was established following the recommendations of the European Society of Cardiology in 2012, 2013, and 2015 and the Orders of the Ministry of Health of Ukraine No. 455 dated July 2, 2014, No. 164 dated March 3, 2016, and No. 152 dated March 2, of 2016.

The study did not include persons older than 75 years, with chronic heart failure of III-IV functional classes according to NYHA, malignant neoplasms, secondary arterial hypertension, acute inflammatory or exacerbation of chronic diseases at the time of inclusion, obesity of II-III degrees, liver and kidney diseases with impaired function, diseases causing secondary dyslipidemia (diabetes, hypothyroidism, nephrotic syndrome, cholestasis).

Endothelial dysfunction markers in the blood serum of patients with CAD were determined by the enzyme-linked immunosorbent assay (ELISA) using special reagent kits (ELISA kits "Endothelin-1" produced by "DRG", USA; "sVCAM" by "Bender Medsystems", Austria; highly sensitive "PAPP-A" "Diagnostics Systems Laboratories", USA).

The average duration of patient observation was 23.1±1.2 months. Contact was lost with 5 patients (2.9 %), and their data were excluded from further analysis. During the observation period, 30 adverse cardiovascular events were noted in 27 of 168 patients (16.1 %), which determined their unfavorable prognosis.

Since the adequacy of therapy largely determines the patient's long-term prognosis, the data of 6 patients (3.57 %) with low compliance were excluded from the analysis of the prognosis criteria. Thus, at the final stage of the study, the data of 135 patients with an uncomplicated course of the disease and 27 with an unfavorable outcome were analyzed.

Excel-2010 spreadsheets and the statistical processing program StatSoft "Statistica" v. 6.0 and 10.0 were used to create a database and analyze the results. The significance of differences was determined

using Student's t-test and Mann-Whitney. To determine independent predictors of the course of atherosclerosis and CAD, a multivariate analysis was performed using multiple stepwise regression ("Multiple Regression" module of the StatSoft "Statistica" v. 6.0 package) and an estimate of the odds ratio (OR).

Results of the study and their discussion. A prospective 2-year study with an analysis of clinical and laboratory indicators in two groups of patients: with and without any unfavorable cardiovascular events, showed that such events were observed significantly more often in the group of patients with coronary heart disease with an initial elevated level of biomarkers of endothelial dysfunction (18.75 %) compared to individuals whose level of these biomarkers was within the reference values (5.0 %).

When comparing clinical data in groups with complicated and uncomplicated coronary artery disease, it was established that the prognosis of patients with coronary artery disease did not significantly depend on the patient's age, sex, smoking, BMI, and the presence of concomitant hypertension (table 1, $p>0.05$). At the same time, the reduction of the left ventricular ejection fraction and the course of coronary heart disease at the beginning of the study had a significant impact on the prognosis ($p<0.01$ and $p<0.05$ correspondingly), with the highest reliability in the group of patients with acute MI. When assessing the impact of MI, which was transferred long before the start of the study, only a trend toward reliability was found in the two-year prognosis ($0.05<p<0.1$).

Table 1

Clinical and laboratory indices in patients with complicated and uncomplicated coronary artery disease (n=162)

Index	Complicated course (n=27)	Uncomplicated course (n=135)	p
Age, y.	59.64±2.62	56.17±3.18	ns
Male sex, n (%)	20 (74.07 %)	98 (72.59 %)	ns
AH, n (%)	13 (48.15 %)	68 (50.37 %)	ns
Smoking pts, n (%)	18 (66.67 %)	70 (51.85 %)	ns
BMI, kg/m^2	29.90±0.76	29.71±0.38	ns
MI history, n (%)	17 (62.96 %)	60 (44.44 %)	<0.1
Revascularization history, n (%)	19 (70.37 %)	70 (51.85 %)	ns
The stable course of the disease, n (%)	9 (33.33 %)	77 (57.04 %)	<0.05
The unstable course of the disease, n (%)	18 (66.67 %)	58 (42.96 %)	<0.05
Acute MI, n (%)	11 (40.74 %)	26 (19.26 %)	<0.05
Unstable stenocardia, n (%)	7 (25.93 %)	32 (23.70 %)	ns
LVH, n (%)	15 (55.56 %)	72 (53.33 %)	ns
EF, %	48.67±1.88	55.69±0.97	<0.01
PAPP-A, mIU/l	12.43±0.47	6.11±0.27	<0.0001
ET-1, ng/ml	11.04±0.37	9.38±0.41	<0.01
sVCAM, ng/ml	1689.3±33.4	1028.3±42.7	<0.0001
Cholesterol, mmol/l	6.02±0.31	5.96±0.06	ns
LDL-C, mmol/l	4.05±0.19	3.97±0.08	ns
HDL-C, mmol/l	1.11±0.03	1.17±0.02	<0.1
TG, mmol/l	1.91±0.04	1.83±0.03	ns
Atherogenity index	4.42±0.48	4.09±0.10	ns

Notes: p – the significance of the difference of parameters between groups with a complicated and uncomplicated course; ns – non-significant difference in parameters ($p>0.05$)

Table 1 shows the initial average levels of PAPP-A and sVCAM were significantly higher in patients with an unfavorable prognosis. The difference in average ET-1 levels between groups was minor but also significant.

Baseline levels of lipid profile parameters in patients with complicated and uncomplicated CAD were not significantly different, excluding HDL cholesterol levels which showed a trend towards significance ($p<0.1$).

The assessment of the odds ratio of clinical and instrumental factors showed that the unstable course of coronary heart disease (OR – 2.655, 95 % CI 1.113–6.556) and, first of all, acute myocardial

infarction history (OR – 2.882, 95 %) had a significant impact on the two-year prognosis (CI 1.197–6.941) and a decrease in left ventricular EF less than 50% (OR – 2.473, 95 % CI 1.052–5.812). Thus, an unfavorable prognosis associated with the occurrence of cardiovascular events was observed almost three times more often in patients who were hospitalized with a diagnosis of acute coronary syndrome and in nearly two and a half times with impaired myocardial contractility, which was probably due to greater severity of the disease in such patients.

When assessing the impact of a previous myocardial infarction and coronary revascularization on the prognosis of the disease, only a trend towards the significance of their influence was found (OR – 2.12, 95 % CI 0.907–4.980 and OR – 2.205, 95 % CI 0.903–5.382, respectively).

A significantly higher probability of unfavorable cardiovascular events was found in patients with high levels of studied biomarkers. When evaluating the parameters of the blood lipid spectrum, only a tendency towards the significance of the association of the HDL-C level <1.1 mmol/l with the prognosis of the disease was revealed. The influence of other parameters of the blood lipid spectrum on the two-year prognosis of the illness was non-significant (table 2).

Table 2

Comparative analysis of the probability of an unfavourable prognosis in patients with CAD during 2 years depending on the level of biomarkers of endothelial dysfunction and blood lipids

Parameter	Cutoff point	OR	95 % CI	χ^2	p
PAPP-A, mIU/l	>6.0	6.40	2.28–17.62	14.975	<0.001
sVCAM, нг/мл	>1300.0	3.35	1.27–8.81	6.480	0.011
ET-1, ng/ml	>9.0	2.87	1.12–7.09	5.095	0.024
Cholesterol, mmol/l	>5.0	1.22	0.51–3.87	0.005	>0.05
TG mmol/l	>3.5	1.28	0.51–2.97	0.212	>0.05
LDL-C, mmol/l	>3.8	1.35	0.57–3.22	0.079	>0.05
HDL-C, mmol/l	<1.1	2.66	1.08–6.51	3.117	<0.1
AI	>4.0	1.16	0.51–2.66	0.178	>0.05

Our data regarding the prognostic significance of markers of endothelial dysfunction in patients with coronary artery disease are consistent with the results of other authors. Thus, according to Mu Wei et al. [9], soluble adhesion molecules - sVCAM-1, sICAM-1, and sE-selectin are significant predictors of CV death in patients with documented CAD, and high levels of sVCAM-1 in patients with advanced angina are associated with an unfavorable prognosis of the disease [12].

There are data that the level of ET-1 in the blood increases significantly in acute myocardial ischemia. Moreover, its content correlates with the severity of the pathological process and prognosis in patients with acute MI and the severity of symptoms in angina pectoris [8, 13].

According to O.P. Shevchenko et al. [3], an increase in the level of PAPP-A has greater sensitivity and specificity for the diagnosis of acute coronary syndrome compared to a rise in the levels of highly sensitive C-reactive protein, interleukin-6, sVCAM-1, and increased levels of PAPP-A (over 10 mIU/l) indicating a less favorable prognosis in patients with coronary artery disease.

Stepwise linear regression analysis confirmed our suggestions. A relationship was noted between the occurrence of adverse CV events and the MI history at the beginning of the study (R^2=0.49; p<0.0001), initial values of left ventricular EF <50.0 % (R^2=0.50; p<0.0001), the level of PAPP-A (R^2=0.53, p<0.0001), and sVCAM (R^2=0.51; p<0.0001). The association of an unfavorable prognosis with the level of ET-1 was somewhat smaller but reliable (R^2=0.29, p<0.05).

The prediction of an unfavorable prognosis based on the correlation and regression analyses using selected risk factors showed a sensitivity of 86 %, a specificity of 80 %, a predicted positive value of 75 %, and a predicted negative value of 96 %.

In our study, high initial levels of sVCAM-1, PAPP-A, and ET-1 in patients with CAD determined a more severe course of the disease but were not associated with markers of myocardial necrosis - the concentration of troponin I and T in the first hours of MI. This gives reason to believe that these biomarkers reflect the instability of the atherosclerotic plaque and can serve as predictors of the acute coronary syndrome. Other authors also support this suggestion [7, 8, 11, 12].

Thus, the levels of sVCAM and PAPP-A can be considered independent predictors of adverse cardiovascular events in the long-term prognosis in patients with coronary artery disease, which in terms of prognostic significance, exceed traditional CV risk factors.

Conclusion

The obtained data give reason to believe that biomarkers of endothelial dysfunction can be considered independent risk factors for adverse cardiovascular events in patients with different clinical courses of CAD.

The presence of MI history, decreased myocardial contractility (left ventricular EF <50%), increased levels of PAPP-A, sVCAM, and, to a lesser extent, ET-1 have a significant predictive value in terms of the probability of adverse CV events.

References

1. Belokopytova IS, Moskalets OV, Paleyev FN, Zotova OV. Diagnosticheskoye znacheniye molekul adgezii sICAM-1 i sVCAM-1 pri ishemicheskoy bolezni serdtsa. Ateroskleroz i dislipidemii. 2013;4(12):62–65 [in Russian]

2. Kovalenko VM, Kornatskyy VM, editors. Aktualni problemy zdorovya ta minimizatsiya yikh v umovakh zbroynoho konfliktu v Ukrayini: posibnyk. Kyiv: Derzhavna ustanova "Natsionalnyy naukovyy tsentr "Instytut kardiolohiyi imeni akademika M.D.Strazheska"; 2018 [in Ukrainian]

3. Shevchenko OP, Shevchenko AO, Kuntsevich NV, Ginzburg LM, Slesareva YuS, Orlova OV. Assotsiirovannyy s beremennostyu protein plazmy A PAPP-A i urovni markerov vospaleniya u bolnykh ishemicheskoy boleznyu serdtsa. Vestnik RGMU. 2010;1:34–40 [in Russian]

4. Byelan OV, Mamontova T, Vesnina L, Borzykh OA, Kaidashev IP. Anti-inflammatory and endothelium protective effect of long-term pioglitazone intake in patients suffering from bronchial asthma concurrent with ischemic heart disease. Wiadomosci Lekarskie. 2017;71(4):712–720.

5. Cacko A, Kondracka A, Gawałko M, Główczyńska R, Filipiak KJ, Bartoszewicz Z, et al. Novel biochemical predictors of unfavorable prognosis for stable coronary disease. Medicine. 2018;97(37). DOI: 10.1097/md.0000000000012372

6. Crea F, Libby P. Acute coronary syndromes. Circulation. 2017;136(12):1155–66. DOI: 10.1161/CIRCULATIONAHA.117.029870

7. Davenport AP, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, et al. Endothelin. Pharmacological Reviews. 2016;68(2):357–418. DOI: https://doi.org/10.1124/pr.115.011833

8. Jankowich M, Choudhary G. Endothelin-1 levels and cardiovascular events. Trends in Cardiovascular Medicine. 2020;30(1):1–8. DOI:10.1016/j.tcm.2019.01.007.

9. Mu W, Chen Mingyou, Gong Z, Zheng F, Xing Q. Expression of vascular cell adhesion molecule-1 in the aortic tissues of atherosclerotic patients and the associated clinical implications. Experimental and Therapeutic Medicine. 2015;10(2):423–8. DOI: 10.3892/etm.2015.2540

10. Nilsson E, Kastrup J, Sajadieh A, Boje Jensen G, Kjøller E, Kolmos H, et al. Pregnancy associated plasma protein-A as a cardiovascular risk marker in patients with stable coronary heart disease during 10 years follow-up – a CLARICOR trial sub-study. Journal of Clinical Medicine. 2020;9(1):265. DOI: 10.3390/jcm9010265

11. Romanova V, Sierkova V, Kuzminova N. P885Levels of soluble vascular cell adhesion molecule-1 and pregnancy-associated plasma protein A as the criteria of coronary heart disease destabilization. European Heart Journal. 2017;38(suppl_1). DOI:10.1093/eurheartj/ehx501.p885

12. Serkova VK, Pavlov SV, Romanava VA, Monastyrskiy YI, Ziepko SM, Kuzminova NV, et al. Medical expert system for assessment of coronary heart disease destabilization based on the analysis of the level of soluble vascular adhesion molecules. SPIE Proceedings. 2017;10445:104453O–8. DOI:10.1117/12.2280984

13. Torres Crigna A, Link B, Samec M, Giordano FA, Kubatka P, Golubnitschaja O. Endothelin-1 axes in the framework of predictive, preventive and personalised (3P) medicine. EPMA Journal. 2021;12(3):265–305. DOI: 10.1007/s13167-021-00248-z

14. Townsend N, Kazakiewicz D, Lucy Wright F, Timmis A, Huculeci R, Torbica A, et al. Epidemiology of Cardiovascular Disease in Europe. Nature Reviews Cardiology. 2021;19(2):133–43. DOI: 10.1038/s41569-021-00607-3

15. Xu S, Ilyas I, Little PJ, Li H, Kamato D, Zheng X, et al. Endothelial dysfunction in atherosclerotic cardiovascular diseases and beyond: From mechanism to pharmacotherapies. Pharmacological Reviews. 2021;73(3):924–67. DOI: 10.1124/pharmrev.120.000096

Стаття надійшла 14.09.2021 р.