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Study of associations of blood proteins with development of unstable atherosclerotic plaques in coronary arteries by quantitative proteomics

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ABSTRACT

Aim. To study the associations of blood proteins with the presence of unstable atherosclerotic plaques in the arteries in patients with coronary artery disease using the quantitative proteomic analysis.

Materials and methods. The study included patients with coronary artery disease (n = 40); the average age of patients was 58 ± 7 years. Material for the study was blood serum. Protein concentrations in serum samples were determined using the PeptiQuant Plus Proteomics Kit (Cambridge Isotope Laboratories, USA). Protein fractions were identified using the liquid chromatograph and tandem mass spectrometer Q-TRAP 6500.

Results. Mass spectrometry revealed an increased concentration of proteins, such as fibrinogen, fibulin-1, and complement factor H, in the serum samples of patients with unstable atherosclerotic plaques. It took place with a simultaneous decrease in the levels of α 2-antiplasmin, heparin cofactor II, coagulation factor XII, plasminogen, prothrombin, vitronectin, complement proteins (C1, C3, C7, C9), and complement factor B. The differences were considered significant at $p < 0.05$. It was revealed that the presence of unstable atherosclerotic plaques was associated with the level of fibulin-1 (Exp(B) = 1.008; $p = 0.05$), plasminogen (Exp(B) = 0.995; $p = 0.027$), and coagulation factor X (Exp(B) = 0.973; $p = 0.037$).

Conclusion. An increased concentration of fibulin-1 can be considered as a potential biomarker of unstable atherosclerotic plaque development in coronary artery disease. The possibility of using the studied proteins as biomarkers of unstable atherosclerotic plaques requires further studies on their potential role in the development of this disease.

Keywords: proteomic analysis, mass spectrometry, coronary artery disease

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at the Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics (Protocol No. 7 of 26.09.2017).

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Изучение ассоциаций белков в крови с наличием нестабильных атеросклеротических бляшек в коронарных артериях методом количественной протеомики

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РЕЗЮМЕ

Цель. Изучение ассоциаций белков крови с наличием нестабильных атеросклеротических бляшек в артериях у пациентов с коронарным атеросклерозом с использованием количественного протеомного анализа.

Материалы и методы. В исследование участвовали пациенты с ишемической болезнью сердца и коронарным атеросклерозом (n = 40), средний возраст пациентов 58 ± 7 лет. Материал исследования – сыворотка крови. Концентрации белков в образцах сыворотки определяли с помощью набора PeptiQuant Plus Proteomics Kit (Cambridge Isotope Laboratories, США). Идентификацию белковых фракций осуществляли методом мониторинга множественных реакций на масс-спектрометре Q-TRAP 6500, комбинированном с жидкостным хроматографом.

Результаты. Масс-спектрометрическая идентификация выявила в образцах сыворотки крови у пациентов с нестабильными атеросклеротическими бляшками повышенную концентрацию белков: фибриноген, фибулин-1 и фактор комплемента H. При одновременном сниженном уровне белков: витронектин, α-2-антиплазмин, кофактор гепарина 2, коагуляционный фактор XII, плазминоген и протромбин, белки комплемента (C1, C3, C7, C9) и фактор комплемента B. Различия считали значимыми при $p < 0,05$. Выявлено, что нестабильность атеросклеротических бляшек ассоциирована с концентрацией фибулина-1 (Exp(B) = 1,008; $p = 0,05$), плазминогена (Exp(B) = 0,995; $p = 0,027$) и коагуляционного фактора X (Exp(B) = 0,973; $p = 0,037$).

Заключение. Повышенная концентрация фибулина-1 в крови может рассматриваться как потенциальный биомаркер нестабильности атеросклеротических бляшек при коронарном атеросклерозе. Возможность использования исследованных белков как биомаркеров нестабильности атеросклеротических бляшек при коронарном атеросклерозе требует дальнейших исследований их потенциальной роли в развитии данного заболевания.

Ключевые слова: протеомный анализ, масс-спектрометрия, коронарный атеросклероз

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Research in the field of etiology and pathogenesis of coronary atherosclerosis, which predetermine complications of this pathology, is currently relevant due to high prevalence and mortality from this disease. Atherosclerosis as the dominant cause of cardiovascular diseases (CVD) includes a number of pathological processes, namely: endothelial dysfunction, excessive lipid deposition in the intima, exacerbations of innate and adaptive immune responses, proliferation of smooth muscle cells, and remodeling of the extracellular matrix, eventually leading to the formation of atherosclerotic plaques (AP).

AP instability aggravates the pathological atherosclerotic process, leading to the development of complications of CVD. The composition of the plaque and internal hemorrhage in the atherosclerotic plaque are independent risk factors for stroke and CAD [1]. Genetically determined inhibition of fibrinogen α-, β- and γ-chains, factor II, and factor XI was associated with a reduced risk of venous thromboembolism ($p < 0.001$). Inhibition of fibrinogen β- and γ-chains was associated with a reduced risk of stroke in large arteries ($p = 0.001$) [2]. Various biomechanical and hemodynamic factors contribute to AP instability. The intact vascular endothelium is thromboresistant, and the damaged endothelium releases increased amounts of procoagulants. Hemostasis is carried out by blood cells and a plasma enzyme system represented by closely interacting protein components. In order to study the involvement of various proteins in the pathogenesis of coronary atherosclerosis, it is necessary to investigate the specific contribution of proteins with pro- or anticoagulation activity to the development of plaque instability in the coronary arteries.

Accumulation of data on the pathogenesis of coronary atherosclerosis and its complications and the development of modern research methods contribute to the search for proteins which may be used as prognostic and diagnostic biomarkers of CVD. A quantitative proteomic analysis which is used for identification and quantification of biological molecules using tandem mass tag mass spectrometry is a useful method in the accurate quantitative simultaneous determination of proteins in various biological samples. Despite the fact that the number of candidate proteins under study is constantly increasing, their role in the pathogenesis of coronary atherosclerosis is not completely clear. Understanding the changes in blood proteins in atherosclerosis will help identify new biomarkers that will give an insight into the conditions underlying the development of complications of this disease.

The aim of this study was to investigate the association of certain blood proteins with unstable AP in the arteries in men with coronary atherosclerosis using quantitative proteomic analysis.

MATERIALS AND METHODS

The study included patients with CAD and coronary atherosclerosis who were referred to coronary artery bypass surgery and who had intraoperative indications for coronary endarterectomy which was carried out during the surgery. Exclusion criteria were as follows: myocardial infarction (less than 6 months ago), acute and chronic infectious and inflammatory diseases or their exacerbations, renal failure, active liver diseases, cancer, hyperparathyroidism. The protocol of the study was approved by the Ethics Committee at the Research Institute of Internal and Preventive Medicine, branch of the Institute of Cytology and Genetics, SB RAS (Protocol No. 7 of 26.09.2017). All patients signed an informed consent to participate in the study.

Blood serum samples were the study material. In all patients, blood was taken from the ulnar vein in the morning on an empty stomach. Blood serum samples of 40 men were selected for the quantitative proteomic analysis. All patient samples were divided into two groups of 20 patients each. Group 1 consisted of patients (average age 58 ± 4 years) with stable AP only, which was determined by the histologic analysis. Group 2 consisted of 20 patients (average age 57 ± 10 years) with unstable AP only, which was also determined by the histologic analysis. We used the PeptiQuant Plus Proteomics Kit (Cambridge Isotope Laboratories, USA) to determine protein concentration in the serum samples according to the method suggested by the manufacturer with some modifications.

The technique of performing trypsinolysis was the following: 20 ml of a solution containing 9 M urea, 20 mM dithiothreitol, and 300 mM Tris-HCl (pH 8.0) was added to 10 ml of a serum sample. We added 10 µl of a bovine serum albumin (BSA) solution to a separate test tube, which was later used as a matrix solution for calibration points. The samples were incubated for 30 min at 37 °C. We added 20 µl of 100 mM iodoacetamide solution to all test tubes. Then the test tubes were incubated for 30 minutes in the dark at room temperature. Later we added 272 µl of

100 mM Tris-HCl (pH 8.0) and 35 µl of a trypsin solution. The test tubes were then incubated for 18 hours at 37 °C. Proteolysis was stopped by adding 343 µl of 2% formic acid.

A mixture of light (unlabeled) peptides was diluted in 60 µl of a solution of 30% acetonitrile and 0.1% formic acid. We prepared a series of dilutions for calibration according to the scheme. A mixture of peptides labeled with heavy stable isotopes was diluted in 450 µl of 30% acetonitrile and 0.1% formic acid and used as an internal standard.

We added 40 µl of serum samples to the test tubes after trypsinolysis and 40 µl of BSA solution after trypsinolysis. Then 10 µl of a labeled peptide solution was added to all tubes. We added 10 µl of diluting standards to the tubes with BSA to create a calibration curve. We added 10 µl of 30% acetonitrile and 0.1% formic acid to the test tubes with serum samples. Then 540 µl of 0.1% formic acid was added to all tubes.

The samples were purified with the Oasis HLB solidphase extraction cartridges (Waters, USA), 10 mg. The cartridges activated 600 µl of methanol and balanced 600 µl of 0.1% formic acid. We took 510 µl of the sample and rinsed it with 600 µl of water 3 times. Peptides were eluted with 300 µl of 50% acetonitrile and 0.1% formic acid. The obtained samples were frozen at –80 °C and dried using the FreeZone 2.5 Dryer designed for lyophilizing light sample loads (Labconco, USA). Dry sediments were diluted in 34 µl of 0.1% formic acid and then used for the analysis.

Peptides were detected by multiple reaction monitoring (MRM) on the Q-TRAP 6500 mass spectrometer (AB Sciex, USA) coupled with the high-performance liquid chromatograph Infinity 1290 (Agilent, USA). Chromatographic separation was carried out on the column Titan C18, 1.9 µm (Supelc, USA) in several stages. The flow rate was 0.4 ml / min, the separation temperature was 45 °C.

Positively charged ions obtained by electrospray ionization in the IonDrive Turbo V Ion Source were detected. We used the Multiquant 3.0.2 software (AB Sciex, USA) to create calibration curves and determine protein concentrations based on the peak area of the MRM transitions specific to each studied peptide.

Statistical data processing was carried out using the SPSS 20.0 software for Windows. The statistical analysis consisted in applying the Kolmogorov – Smirnov test and the Mann – Whitney *U* test to normally distributed data. The age of patients was presented as the mean and the standard root-mean-square deviation ($M \pm \sigma$). The results in the table were presented as the median and the interquartile range (Me [Q_{25};Q_{75}]). A multivariate logistic regression analysis was carried out to determine associations. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Proteomic profiling of the blood serum samples was performed using the PeptiQuant Plus Proteomics Kit. A total of 125 proteins were identified. The identification of proteins was carried out by MRM using a triple quadrupole ultra-high resolution time-of-flight mass spectrometer with electrospray ionization coupled with a high-performance liquid chromatograph.

The differential protein expression analysis was carried out by two technical replicates for each sample. Following the comparative analysis, we isolated proteins, the concentration of which had a statistically significant difference in the study groups ($p < 0.05$).

Table

Quantitative mass spectrometry-based protein identification in the blood, Me [Q_{25};Q_{75}]

Protein	Protein concentration, fmol / µl		*p*
	Group 1	Group 2	
Fibrinogen, α-chain	143.5 [139.4;147.7]	254.4 [243.6;284.9]	0.006
Fibrinogen, γ-chain	45.9 [40.9;52.1]	120.9 [45.4;165.8]	0.005
Fibulin-1	663.9 [576.1;748.6]	735.2 [600.4;796.0]	0.038
Fibronectin	429.6 [234.0;522.5]	256.2 [197.9;402.3]	0.161
Thrombospondin-1	75.0 [63.0;95.2]	75.1 [53.8;89.8]	0.419
Vitronectin	2,839.5 [2,166.2;3,362.2]	2,151.0 [1,654.0;2,878.0]	0.005
α2-antiplasmin	522.2 [427.2;649.4]	472.2 [323.4;599.2]	0.034
α2-macroglobulin	4,885.0 [4,342.5;5,345.5]	4,714.5 [3,324.5;5,868.2]	0.376
Antithrombin III	3,880.0 [2,460.75;4,845.75]	3,272.5 [3,017.5;4,291.0]	0.844
Heparin cofactor II	4,274.5 [4,057.7;4,577.5]	3,803.0 [3,055.0;4,233.0]	0.0001
Coagulation factor IX	87.8 [67.7;107.9]	74.1 [48.2;124.0]	0.166
Coagulation factor X	136.0 [107.5;142.4]	105.3 [92.3;123.3]	0.065

Table (continued)

Protein	Protein concentration, fmol / µl		*p*
	Group 1	Group 2	
Coagulation factor XII	419.4 [294.9;525.0]	291.1 [265.8;380.6]	0.0001
Kininogen-1	178.3 [162.5;205.7]	175.5 [154.9;192.1]	0.346
Complement component C1q, subunit B	67.1 [53.3;95.9]	68.5 [57.2;80.2]	0.538
Complement component C1q, subunit C	106.2 [93.8;143.3]	112.1 [92.4;146.5]	0.939
Complement component C1r	251.2 [169.7;273.6]	194.0 [164.2;242.5]	0.106
Complement component C1s	47.4 [42.8;54.0]	43.8 [34.0;74.8]	0.729
Complement Component C3	586.8 [469.1;717.1]	471.8 [408.7;572.0]	0.008
Complement component C7	75.9 [56.4;82.0]	60.4 [54.2;74.9]	0.006
Complement component C9	191.3 [113.9;212.5]	137.7 [75.4;185.5]	0.026
Complement factor B	4,985.0 [3,585.0;6,251.2]	3,980.5 [3,698.0;4,358.7]	0.017
Complement factor H	526.4 [463.3;587.8]	581.4 [531.9;626.6]	0.018
Plasma protease C1 inhibitor	2,037.0 [1,565.0;2,294.0]	1,651.5 [1,092.0;2,234.7]	0.041
Plasma serine protease inhibitor	51.4 [48.2;62.1]	49.4 [46.5;74.6]	0.769
Plasminogen activator inhibitor-1	27.6 [21.1;36.4]	24.1 [19.2;32.1]	0.102
Plasminogen	933.1 [833.2;1,050.5]	803.5 [695.6;879.2]	0.001
Prothrombin	902.2 [711.1;1,044.2]	788.4 [718.7;821.1]	0.047

Fibrinogen, which is one of the main proteins in the coagulation system, differed significantly in the study groups. In the group of patients with unstable plaques, the concentration of each of the two fibrinogen isoforms (α- and γ-chain) was 1.8 and 2.5 times higher, respectively (Table). At the same time, the level of fibulin which binds to fibrinogen and incorporates into clots, was also higher in the group of patients with unstable plaques. In addition, the multivariate logistic analysis showed that the instability of atherosclerotic plaques was associated with the concentration of fibulin-1 (Exp(B) = 1.008; 95% confidence interval (CI) 1.000–1.015; $p = 0.05$).

In our study, the level of plasminogen, which is the main component of the fibrinolytic system, was higher in the group of patients with stable plaques ($p < 0.05$). Also, the multivariate logistic analysis showed that the instability of atherosclerotic plaques was reversely correlated with the levels of plasminogen (Exp(B) = 0.995; 95% CI 0.990–0.999; $p = 0.027$), heparin cofactor II (Exp(B) = 0.999; 95% CI 0.998–1.000; $p = 0.010$), and coagulation factor X (Exp(B) = 0.973; 95% CI 0.949–0.998; $p = 0.037$). Fibrinolytic activity of the blood also depends on fibrinolysis inhibitors. The concentration of plasminogen activator inhibitor-1 (PAI-1) was 13% higher in the group of patients with stable plaques, but did not reach the level of statistical significance ($p = 0.102$).

In addition, the concentration of proteins, primary anticoagulants (α2-antiplasmin, α2-macroglobulin, heparin cofactor II), was significantly higher in patients with stable plaques (Table). At the same time, the level of the anticoagulant antithrombin III was higher in the blood of patients with unstable plaques. Antithrombin III is a universal inhibitor of thrombin and almost all clotting factors, which is confirmed by our study; the levels of coagulation factors IX, X, and XII were lower in group 2.

At the same time, the level of coagulation factor XII was significantly higher in the group of patients with stable plaques. Highly activated factor XII in combination with kininogen activates fibrinolysis. In our study, the level of kininogen was higher in the group of patients with stable plaques, but it did not reach the level of statistical significance.

Blood clotting mechanisms are linked with the activation of the immune system. In our study, there was no significant difference between the C1 (C1q; C1r; C1s) complement components in the study groups (Table). However, the total level of the complement component C1 was higher in the group of patients with stable AP and amounted to 471.37 fmol / µl vs. 446.48 fmol / µl, compared with the group of patients with unstable AP ($p < 0.0001$). The content of complement components C3, C7, C9, and complement factor B was higher in the group of patients with stable AP ($p < 0.05$). The level of the complement factor H involved in C3b inactivation was 10% higher in the group of patients with unstable AP (Table).

Fibronectin, thrombospondin, and vitronectin are also involved in the coagulation cascade, promoting platelet adhesion. In our study, no significant difference was revealed in the content of fibronectin and thrombospondin between the study groups. The

concentration of vitronectin was significantly higher in patients with stable plaques ($p = 0.005$).

DISCUSSION

Atherosclerosis is associated with inflammation and vascular endothelial dysfunction. The intact vascular endothelium is thromboresistant, and the damaged endothelium releases increased amounts of procoagulants.

In our study, the levels of fibrinogen and fibulin-1, which is related to it, differed significantly in the study groups. The concentrations of each of the two fibrinogen isoforms (α- and γ-chain) and fibulin-1 were higher in the group of patients with unstable plaques. The multivariate logistic analysis showed that AP instability was associated with the concentration of fibulin-1. Previously, we found that the maximum amount of fibrinogen was in the tissue of stable fibrous AP, and the protein level was slightly lower in unstable AP [3]. Thus, the study suggests that high concentrations of fibrinogen and fibulin-1 may be a promising biomarker of AP instability in the blood of patients with coronary atherosclerosis.

Fibronectin, thrombospondin, and vitronectin are involved in the coagulation cascade. Platelet activation caused a local release of fibrinogen, fibronectin, vWF, thrombospondin, vitronectin, and clotting factors, promoting platelet adhesion and increased coagulation [4]. In our study, no significant difference was revealed in the content of fibronectin and thrombospondin between the study groups.

Vitronectin is the main glycoprotein of cell adhesion contained in plasma and extracellular matrix. Increased expression of vitronectin may contribute to the development of chronic vascular diseases, such as atherosclerosis, playing an important role in vascular homeostasis and pathological vascular remodeling. Plasminogen activator inhibitor-1 (PAI-1) stimulated vitronectin expression by binding low-density protein receptor-related protein-1 (LRP1). The concentration of vitronectin in the blood plasma was significantly reduced in mice with PAI-1 deficiency compared with the control [5]. When binding to PAI-1, vitronectin participates in the activation of fibrinolysis. In atherosclerosis of the carotid arteries, it was shown that the level of PAI-1 in blood plasma was reduced in the experimental group compared with the controls. At the same time, the level of PAI-1 was positively correlated with the level of vitronectin in the group of patients with atherosclerosis, which may be due to increased fibrinolytic activity and disease progression, promoted by increased vascular remodeling [6]. The level of vitronectin was significantly higher in the blood of patients with CAD than in the control group. Vitronectin is assumed to be a marker of CAD [7]. In our study, the concentration of fibronectin in the blood was significantly higher in patients with stable plaques.

Earlier, a prospective cohort study revealed that high levels of factors IX and XI and α2-antiplasmin were associated with an increased risk of CAD, and did not depend on other coronary risk factors [8]. Correlations between the level of factor XII in the blood and the presence of vulnerable atherosclerotic plaques in the coronary arteries were shown [9].

In our study, the concentrations of proteins α2-antiplasmin, α2-macroglobulin, heparin cofactor II, and coagulation factor XII were significantly higher in patients with stable plaques than in group 2. Antithrombin III (AT-III) is a universal inhibitor of thrombin and almost all clotting factors, but in our study, the concentration of antithrombin III in the blood was higher in patients with unstable plaques, although the level of statistical significance was not reached.

The levels of coagulation factors IX, X, and XII were lower in group 2. At the same time, the logistic regression analysis showed that the instability of AP was negatively correlated with the concentrations of coagulation factor X, heparin cofactor II, and plasminogen.

Previously, it was shown that the level of AT-III was reduced in the high-risk subgroup of acute coronary syndrome (ACS) compared with the control group ($p < 0.05$). The logistic regression model demonstrated that AT-III was a protective factor (odds ratio (OR) = 0.958; $p = 0.012$) for ACS. The level of AT-III demonstrated prognostic value in patients with ACS and was associated with the severity of CAD [10]. At the same time, a reduced level of antithrombin III is not an independent risk factor for myocardial infarction [11].

Highly activated factor XII in combination with kininogen activates fibrinolysis. Kininogen is a precursor of bradykinin and kallidin, proteins that cause vasodilation and smooth muscle contraction. Besides the fact that kinins are known for their ability to induce nitric oxide and prostacyclin, which mediate cardioprotection, bradykinin promotes inflammation, fibroplasia, and fibrosis after myocardial infarction in rats [12]. Thus, kininogen is involved in inflammation, blood pressure control, coagulation, and emergence

of pain. With kininogen deficiency, the level of bradykinin decreases, which does not affect the function of the left ventricle, but affects the risk of CAD [13]. In our study, the level of kininogen did not differ in the study groups. However, high levels of plasminogen and fibrinolysis inhibitor PAI-1 were found in the group of patients with stable plaques.

There is a relationship between the activation of the immune system, inflammation, and blood clotting mechanisms. The complement system is not only a component of the innate immune response, but also a key mediator of inflammation [14]. Complement system proteins have been repeatedly associated with vascular remodeling and atherosclerosis [15, 16].

Research data indicate that the anomaly of complement components and the resulting excessive complement activation are associated with atherogenesis. C3b / iC3b and MAC deposition in the clogged arteries indicates increased complement activation [17]. The complement system can modulate platelet activation and subsequent formation of blood clots. It was reported that several components of the complement system, including C3 and the membrane attack complex, are associated with platelets and become functionally active when platelets are activated [18]

The complement system can be activated through three pathways: classical, lectin, and alternative. All three pathways converge at the C3 level to form the C5 convertase, which ultimately leads to the polymerization of C9 and the formation of membrane attack complexes. The classical pathway is triggered by the recognition of C1q antibodies or apoptotic cells associated with antigens or microbial surfaces. Exposure of C1q to its target results in activation of serine proteases C1r and C1s, followed by C1s-mediated cleavage of C4 into anaphylatoxin C4a and opsonin C4b. [14, 18]. Platelets can also release C1q upon activation, thereby activating other platelets [19]. There are several points of interaction in the complement cascade and the blood coagulation system. For example, factor XIIa can activate C1q and, consequently, the classical complement pathway [20].

In our study, no significant difference was found between the C1 (C1q; C1r; C1s) complement components in the study groups. However, the total content of the complement component C1 was higher in the group of patients with stable AP. The levels of complement components C3, C7, C9 and complement factor B were higher in the group of patients with stable AP ($p < 0.05$) (Table). The level of complement factor H involved in C3b inactivation was higher in the group of patients with unstable AP (Table).

Earlier ELISA studies showed high levels of C5b-9 in intimal thickening and fibrous plaques compared with normal tissue. At the same time, the levels of C5b-9 in intimal thickening were higher than in fibrous plaques, which allowed the authors to assume that complement activation occurs directly in the artery wall and plays an important role in atherogenesis [16].

Inflammation and activation of the C5b-9 complement system predispose to rupture of an intracranial aneurysm [21]. In an animal experiment, it was shown that the complement factor C5a and its receptor C5aR are expressed in vulnerable atherosclerotic plaques. A significant increase in C5aR in the plaque was found in mice treated with C5a, while local treatment with C5a led to a significant increase in plaque destruction with concomitant bleeding. In addition, the authors demonstrated that smooth muscle cells and endothelial cells after C5a treatment *in vitro* showed a marked increase in apoptosis, which may contribute to the instability of the lesion *in vivo* [22]. It was also found that the membrane attack complex can play a crucial role in the formation of plaques and rupture of aneurysms [23]. In our study, the concentration of complement components C7 and C9 involved in the formation of the membrane attack complex did not increase in the blood of patients with unstable plaques. Apparently, this process is local in the tissue of the atherosclerotic plaque.

CONCLUSION

The possibility of using the studied proteins as biomarkers of AP instability in coronary atherosclerosis requires further research devoted to their potential role in the development of this disease. The data of this study, obtained using a modern method of quantitative proteomics, revealed elevated concentrations of complement factor H, fibrinogen, and fibulin-1 in the blood serum samples of patients with unstable AP. When the levels of proteins which are involved in the coagulation cascade and fibrinolysis and proteins related to them functionally (α2-antiplasmin, α2-macroglobulin, heparin cofactor II, coagulation factor XII, prothrombin, plasminogen, PAI-1, vitronectin) reduced simultaneously, the concentrations of complement proteins (C1, C3, C7, C9) and complement factor B associated with coagulation and fibrinolysis were also found to be reduced in the group of patients with unstable AP.

Our data showed that the inhibition of coagulation and fibrinolysis in the blood of patients with unstable AP significantly increased the concentrations of fibulin-1 and fibrinogen. This was confirmed by the multivariate logistic regression analysis, which showed the relationship of instability with the concentration of fibulin-1 (Exp(B) = 1.008; $p = 0.05$).

Thus, an increased concentration of fibulin-1 in the blood may be considered as a promising potential biomarker of AP instability in coronary atherosclerosis.

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