АССОЦИАЦИЯ ФАКТОРОВ СВЕРТЫВАНИЯ КРОВИ С НАЛИЧИЕМ НЕСТАБИЛЬНЫХ АТЕРОСКЛЕРОТИЧЕСКИХ БЛЯШЕК В КОРОНАРНЫХ АРТЕРИЯХ

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Цель. Настоящее исследование было посвящено изучению некоторых факторов свертывания крови (фактор II, фактор VII, фактор XII, антитромбин III) с целью поиска их ассоциаций с биомаркерами эндотелиальной дисфункции (эндотелин 1, моноцитарный хемоаттрактантный протеин 1 типа, моноцитарный хемоаттрактантный протеин 1 типа, липопротеин(а), адгезивные молекулы sVCAM-1, ассиметричный диметиларгинин, гомоцистеин), воспаления (интерлейкины, интерлейкин-6, интерлейкин-8, С-реактивный протеин) и с наличием нестабильных атеросклеротических бляшек в коронарных артериях у мужчин с коронарным атеросклерозом.

Материал и методы. У 93 мужчин с коронарным атеросклерозом без острого коронарного синдрома исследовали в крови концентрации факторов свертывания крови (фактор II, фактор VII, фактор XII, антитромбин III) с целью поиска их ассоциаций с биомаркерами эндотелиальной дисфункции (эндотелин 1, моноцитарный хемоаттрактантный протеин 1 типа, липопротеин(а), адгезивные молекулы sVCAM-1, ассиметричный диметиларгинин, гомоцистеин), воспаления (интерлейкин-6, интерлейкин-8, С-реактивный белок) и с наличием нестабильных бляшек в коронарных артериях.

Результаты. У мужчин с наличием нестабильных атеросклеротических бляшек в коронарных артериях содержание в крови фактора VII и фактора XII было выше в 1,3 и 1,3 раза, соответственно, в сравнении с мужчинами, у которых в коронарных артериях были стабильные бляшки. Выявлены корреляционные связи между содержаниями в крови фактора II и фактора XII и сналичием у пациентов нестабильных атеросклеротических бляшек в коронарных артериях (r=0,239 и r=0,250, p<0,05, соответственно), а также между факторами свертывания крови и уровнями в крови липопротеина(а), адгезивные молекулы sVCAM-1, интерлейкина-6 и С-реактивный белок. Результаты логистического регрессионного анализа показали, что относительный риск наличия в коронарных артериях нестабильных атеросклеротических бляшек связан с повышенным уровнем в крови фактора XII (OR=1,008, 95% CI 1,000-1,017, p=0,048).

Заключение. Полученные нами результаты свидетельствуют о том, что повышенный уровень в крови фактора Хагемана может быть новым биомаркером вероятности наличия нестабильных атеросклеротических бляшек в коронарных артериях.

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ADMA — asymmetric dimethylarginin, CRP — C-reactive protein, IL — interleukins, LP(a) — lipoprotein (a), MCP-1 — monocytic chemoattractant protein type 1.

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ASSOCIATION OF COAGULATION FACTORS WITH THE PRESENCE OF UNSTABLE ATHEROSCLEROTIC PLAQUES IN CORONARY ARTERIES

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Aim. The study was aimed to explore some blood coagulation factors (factor II, factor VII, factor XII, antithrombin III) in order to find their associations with biomarkers of endothelial dysfunction (endothelin 1, monocytic chemoattractant protein type 1, MCP-1, lipoprotein (a), LP (a), adhesive molecules sVCAM-1, asymmetric dimethylarginin, ADMA, homocysteine), inflammation (interleukins, IL-6, IL-8, C-reactive protein, CRP) and with unstable atherosclerotic plaques in coronary arteries in men with coronary atherosclerosis.

Material and methods. In 93 men with coronary atherosclerosis with no acute coronary syndrome, blood coagulation factors concentrations (factor II, factor VII, factor XII, antithrombin III) were studied in the blood with the aim to find associations with biomarkers of endothelial dysfunction (endothelin 1, MCP-1, LP(a), adhesion molecules sVCAM-1, ADMA, homocysteine), of inflammation (IL-6, IL-8, CRP) and with the presence of unstable plaques in coronary arteries.

Results. In men with unstable atherosclerotic plaques in coronary arteries, blood levels of factor VII and factor XII were 1,3 and 1,3 times higher, respectively, compared to men who had stable plaques in coronary arteries. Correlations between the blood levels of factor II and factor XII and presence of unstable atherosclerotic plaques in coronary arteries (r=0,239 and r=0,250, p<0,05, respectively) have been found, as well as between coagulation factors and blood levels of LP(a), sVCAM-1, IL-6 and CRP. Results of logistic regression analysis showed that the relative risk in presence of unstable atherosclerotic plaques in the coronary arteries is associated with an elevated blood level of factor XII (OR=1,008, 95% CI 1,000-1,017, p=0,048).

Conclusion. Our results indicate that elevated blood levels of Hageman factor may be a new biomarker of probability of unstable atherosclerotic plaques presence in coronary arteries.

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Prevalence and mortality from acute coronary syndrome (ACS) and myocardial infarction remain high in Russia. The trigger for the clinical manifestations of ACS is an erosion of the integrity of the endothelium at the site of ulceration/destruction of unstable atherosclerotic plaque cover and subsequent thrombus formation and artery occlusion, ischemia and necrosis of the myocardium [1]. Stable plaque is characterized by a thick cover, homogeneous lipid core, the absence of inflammatory changes, and unstable — by thin cover (thickness <65 μm), or section of thinned cover with focal destruction of the endothelium, inflammatory cell infiltration (more than 25 cells per field of view with a length of 0,3 mm), loose lipid core (>40% of plaque volume) with areas of necrosis [2].

It is known that at the initial stage of atherosclerotic plaque development endothelial dysfunction and oxidative changes play an important role, while at the stage of unstable plaque development the activity of inflammatory-destructive processes is pronounced [1, 3-5]. At the same time, it is known that disorders of hemostasis accompany almost all stages of atherosclerotic focus development. Components of the hemostatic system not only participate in thrombosis of the affected areas of blood vessels, but also can play an important role in the process of formation and progression of atherosclerotic stenosis [5, 6].

In recent years, many studies have been carried out to find and study various etiopathogenetic biomarkers of coronary atherosclerosis and its complications, especially ACS [7-9]. This study was devoted to examination of some factors of blood coagulation (factor II, factor VII, factor XII, antithrombin III) in order to find their associations with biomarkers of endothelial dysfunction (endothelin 1, monocytic chemoattractant protein type 1, MCP-1, lipoprotein (a), LP (a), adhesive molecules sVCAM-1, asymmetric dimethylarginin, ADMA, homocysteine), inflammation (interleukins, IL-6, IL-8, C-reactive protein, CRP) and with unstable atherosclerotic plaques in the coronary arteries in men with coronary atherosclerosis.

Material and methods

The study was conducted under the framework of combined study of Research Institute of Internal and Preventive Medicine — Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences and The Federal State Budgetary Institution "National Medical Research Center named after academician E. N. Meshalkin" of the Ministry of Health of the Russian Federation. The study was approved by the Ethics committees of both institutions. The study was performed in the framework of the budget theme of the State assignment № 0324-2018-0002, budget theme in support of bioresource collections of the State assignment № 0324-2017-0048 and with the financial support of RFBR Grant No. 17-04-02120a.

The study included 93 men 40-70 years of age with coronary angiographic verified coronary atherosclerosis, without ACS with stable angina II-III FC admitted to the Clinic of the FSBI "National Medical Research Center named after academician E. N. Meshalkin" of the Ministry of Health of the Russian Federation on coronary bypass surgery, which during surgery for intraoperative indications was performed endarterectomy from coronary artery/arteries. Material from endarterectomy containing the intima/media of the artery was transversely divided into fragments, containing atherosclerotic plaque for histological studies. Histological analysis of fragments of the intima/media of the coronary arteries after standard hematoxylin-eosin and van Gison staining was carried out on a binocular microscope Axiostar Plus (C. Zeiss) with a digital photo output. Stable and unstable atherosclerotic plaques differentiated according to the criteria described above. According to the histological conclusion, 38 men (41%) had only stable atherosclerotic plaques in coronary arteries (CA), and 55 men (59%) also had unstable plaques in CA along with stable plaques. According to this criterion, all examined patients were selected to 2 groups.

For biochemical research with enzyme immunoassay (ELISA) using standard test systems ELISAs on the analyzer Multiscan EX (Finland) before coronary artery bypass surgery all the men one-shot after an overnight fast were carried out blood sampling from a vein to obtain plasma and serum. In the blood plasma was determined following clotting factors: factor II, factor VII, factor XII, antithrombin III (test system AssayPro). Endothelial dysfunctional and inflammatory biomarkers were determined in serum: endothelin 1 (Biomedica), monocytic chemoattractant protein type 1, MCP-1 (Bender Medsystems), lipoprotein (a), LP(a) (AssayPro), adhesive molecules sVCAM-1 (Biosource), asymmetric dimethylarginin, ADMA (Immunodiagnost),

Table 1

Factors of hemostasis and endothelial function in men with coronary atherosclerosis (M±σ)

Factors of hemostasis and endothelial dysfunction in the blood	Men (n=38) with stable plaques in the coronary arteries	Men (n=55) with unstable plaques in the coronary arteries	P
Factor II, μg/ml	250,5±55,5	261,5±57,5	0,134
Factor VII, ng/ml	420,5±143,5	553,9±141,0*	0,046
Factor XII, μg/ml	85,7±57,7	114,7±54,3*	0,016
Antithrombin III, μg/ml	611,4±185,9	598,4±227,7	0,771

Note: * — p<0,05.

Table 2

Correlations of hemostatic factors with factors of endothelial dysfunction (ED), inflammatory factors and indicators of instability of atherosclerotic plaques in coronary arteries (r Spearman)

Factors of hemostasis Factors ED and inflammation	Factor II	Factor VII	Factor XII	Antithrombin III
Endothelin 1, fmol/ml	p>0,05	p>0,05	p>0,05	p>0,05
MCP1, pg/ml	p>0,05	p>0,05	p>0,05	p>0,05
sVCAM-1, ng/ml	p>0,05	0,347*	p>0,05	p>0,05
LP(a), mg/dl	-0,821*	0,812*	p>0,05	p>0,05
ADMA, ng/ml	p>0,05	p>0,05	p>0,05	p>0,05
Homocysteine, μmol/l	p>0,05	p>0,05	p>0,05	p>0,05
IL-6, pg/ml	0,235*	p>0,05	p>0,05	-0,355**
IL-8, pg/ml	p>0,05	p>0,05	p>0,05	p>0,05
CRP, mg/l	p>0,05	p>0,05	0,274*	p>0,05
The presence of unstable plaques in CA	0,239*	p>0,05	0,250*	p>0,05

Note: * — p<0,05, ** — p<0,01

Abbreviation: CA — coronary arteries.

homocysteine (Ahis-Shield), interleukins, IL-6, IL-8 (Bender Medsystems), C-reactive protein, CRP (Biomerica).

Statistical processing of the results was carried out in the licensed version of SPSS for Windows with the use of correlation, logistic regression and One-Way ANOVA analyses using The Dunnet criteria for multiple comparisons.

Results

In men with unstable atherosclerotic plaques in coronary arteries, plasma levels of factor VII and factor XII (Hageman factor) were both 1,3 higher (p<0,05), compared with men who, according to the histological conclusion on intima/media samples, did not have unstable plaques in the coronary arteries (Table 1). There were no differences between the two groups of men in plasma levels of factor II and antithrombin III.

The results obtained do not contradict the data of other studies devoted to the study of coagulation factors VII and XII in atherosclerosis. Thus, according to the research of Cirillo P. and co-authors, activation of the external blood clotting pathway by binding tissue factor with circulating factor VII plays a crucial role in the development of endothelial dysfunction and atherosclerosis progression. There was a positive and independent link between the activity of coagulation factor VII and cardiovascular events [10]. Renne T, et al. believe that the Hageman factor — one of the key factors in the formation of fibrin-is a biomarker of atherosclerotic vascular damage [11].

The results of the correlation analysis of the studied hemostasis factors and atherosclerosis biomarkers in the blood, taking into account the nonparametric distribution of features is presented in Table 2. Revealed correlation of blood levels of factor II (prothrombin) concentration of LP(a) (strong negative relationship) and IL-6 (weak positive relationship); the positive correlation of factor VII with LP(a) (strong positive relationship) and sVCAM-1; a weak correlation between the Hageman factor and CRP and a negative correlation between antithrombin III and IL-6. In addition, blood levels of coagulation factors II and XII are positively weakly correlated with the presence in men unstable atherosclerotic plaques in the CA.

Combination of inflammatory process with hemocoagulation disorders is characteristic of atherogenesis. It is known that the Hageman factor involved in two important biological processes — coagulation and kinin formation — active mediators of inflammation, so it is the link between inflammation and coagulation [12, 13]. Therefore, revealed correlation between the Hageman factor and the inflammatory marker CRP is quite understandable. We have identified the correlation of prothrombin with inflammatory marker IL-6 can also be explained because of Hageman factor

Table 3

Logistic regression analysis of the relative risk of unstable atherosclerotic plaques presence in coronary arteries associated with hemostasis factors

Factors of hemostasis	Exp(B)	95,0% C.I. for Exp(B)		P
		Lower	Higher	
Factor II	1,001	0,993	1,009	0,832
Factor VII	1,000	1,000	1,001	0,667
Factor XII	1,008	1,000	1,017	0,048
Antithrombin III	1,000	0,998	1,002	0,797

activates prothrombin by activation of a plasma thromboplastin precursor. In addition, the results obtained confirm the data of Miller GJ, et al, who also revealed the correlation of CRP with several markers of blood coagulation activation, the largest-with the factor XII [14].

All studied factors of blood clotting were included in the model of logistic regression analysis (Table 3). The results showed that the relative risk of unstable atherosclerotic plaques in the coronary arteries is associated only with an increased level of factor XII (OR=1,008, 95% CI 1,000-1,017, p=0,048).

Kuijpers MJ, et al. who studied the accumulation of factor XIII on the external surface of blood clots by means of their immunological staining believe that factor XII regulates the pathological process of thrombosis on the surface of atherosclerotic plaques complicated by rupture [15].

Conclusion

The results obtained, firstly, on the correlation of blood plasma levels of factors II and XII with the presence in men with coronary atherosclerosis histologically verified unstable plaques in coronary arteries and, secondly, on the link of elevated blood levels of factor XII with the relative risk of unstable atherosclerotic plaques presence in coronary arteries, not only do they not contradict the literature data, but also showed that elevated blood Levels of the Hageman factor may be a new biomarker of probability of unstable atherosclerotic plaques presence in the coronary arteries.

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