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Platelet adhesion mediated by von Willebrand factor in patients with premature coronary artery disease

Aim	To study platelet adhesion mediated by von Willebrand factor (VWF) in patients with premature ischemic heart disease (IHD).
Material and Methods	This study enrolled 58 patients with stable IHD, including 45 men younger than 55 years with the first manifestation of IHD at the age of <50 years and 13 women younger than 65 years with the first manifestation of IHD at the age of <60 years. The control group consisted of 33 patients, 13 men younger than 55 years and 20 women younger than 65 years without IHD. Platelet adhesion to the collagen surface at the shear rate of 1300 s-1 was studied by evaluating the intensity of scattered laser light from the collagen-coated optical substrate in a flow chamber of a microfluidic device after 15-min circulation of whole blood in the chamber. Decreases in platelet adhesion after addition to the blood of monoclonal antibodies (mAb) to platelet receptors glycoproteins Ib (GPIb) to inhibit the receptor interaction with VWF were compared for patients of both groups.
Results	In patients with premature IHD, the decrease in platelet adhesion following the platelet GPIb receptor inhibition was significantly less than in patients of the control group (74.8% (55.6; 82.7) vs. 28.9% (–9.8; 50,5), p <0.001). For the entire sample, the median decrease in platelet adhesion following the GPIb receptor inhibition was 62.8% (52.2; 71.2). With an adjustment for traditional risk factors of IHD, a decrease in platelet adhesion of >62.8% after blocking GPIb receptors increased the likelihood of premature IHD (OR=9.84, 95% CI: 2.80–34.59; p <0.001).
Conclusion	Blocking the interaction of GPIb receptors with VWF in patients with premature IHD and increased shear rate induced a greater decrease in platelet adhesion than in patients without this disease. This suggested that an excessive interaction of VWF with platelets might contribute to the pathogenesis of premature IHD.

Keywords	Von Willebrand factor; platelet adhesion; ischemic heart disease
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Introduction

The likelihood of coronary artery disease (CAD) increases with age. However, it can develop in young people, and myocardial infarction (MI) is often its first manifestation [1]. Premature CAD, which is CAD that occurred before the age of 55 in men or before the age of 65 in women, is an aggressive disease that often leads to early death and adverse cardiovascular events [2]. The development of occlusive coronary artery thrombosis, which causes MI, depends on the state of the hemostasis system. Occlusive thrombosis is obviously more likely in people prone to clotting. Given the often-acute onset and the aggressive course of premature CAD, we suggested that such events may be associated with the peculiarities of parietal thrombosis at high shear rates characteristic of stenotic arteries. Von Willebrand factor (vWF) is a key factor of the hemostasis system involved in the clotting process. The objective of this study was to investigate vWF-mediated platelet adhesion to the collagen surface at high shear rates in patients with premature CAD.

Material and methods

Subjects

The study included 58 patients with stable CAD, including 45 men younger than 55 years, with CAD manifestation before the age of 50 years, and 13 women younger than 65 years, with CAD manifestation before the age of 60 years, who had stenotic

coronary artery lesions according to coronary angiography (CAG). The control group consisted of 33 patients: 13 men < 55 years old and 20 women <65 years old without clinical manifestations of CAD and stenotic coronary atherosclerosis in CAG and/or computed tomography angiography of coronary arteries when CAD is suspected. Indications for CAG and/or computed tomography angiography of coronary arteries were determined in patients of the control group by their attending physicians. A stenotic lesion of the coronary arteries was a lesion that caused a decrease by 50% or more in the left coronary artery lumen, and/or a main coronary artery (left anterior descending artery, left circumflex artery, right coronary artery), and/or a second-order branch with a diameter of >2 mm [3].

Patients with familial hypercholesterolemia, low-density lipoprotein (LDL) cholesterol >4.9 mmol/L, unstable angina pectoris, the first two months after MI, coronary artery bypass grafting or angioplasty, heart failure NYHA functional class III–IV, left ventricular ejection fraction < 40%, persistent atrial fibrillation/flutter, aortic valve stenosis or left atrioventricular stenosis, hereditary and acquired coagulopathies, malignancies, clinical and laboratory signs of acute infectious disease in the previous two months, were excluded.

The study protocol was approved by the ethics committee of the Academician Chazov Russian National Medical Research Center (Minutes No. 262 dated 30/11/20) and conducted following the 1964 Declaration of Helsinki. All patients signed the informed consent.

Material

The optical substrate was incubated with a collagen solution 0.1 mg/mL on its surface at room temperature for 2 hours. The glass surface of the optical substrate was treated before coating with collagen with a 70% solution of ethyl alcohol. Rat collagen type I and phosphate-buffered saline (Sigma, USA), rabbit monoclonal antibodies (mAbs) specific to human platelet GPIb receptors (IMTEK, RF) were used in the study. Collagen solutions were stored at +4°C, mAb were kept at –70°C.

Collection of whole blood samples

Blood was collected from the cubital vein into S-Monovette vacuum tubes (Sarstedt, Germany) containing D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone 100 μM (Enzo, USA). All experiments were conducted within 2 hours after blood sampling.

Measurement of platelet adhesion to collagen surface

A microfluidic device was designed in the laboratory of cell hemostasis of the Institute of Experimental Cardiology of the Academician Chazov Russian National Medical Research Center. The device was intended for recording the adhesion kinetics of blood cells to the protein surface under conditions of controlled flow [4, 5]. The device consists of a flow chamber with a collagen-coated optical substrate, a peristaltic pump that provides blood flow through the flow chamber, a laser source, a photodetector, and an analog-to-digital converter connected to a computer (Figure 1). At the first stage of the experiment, whole blood was placed in a microtube to be connected to a system ensuring the movement of blood in the flow chamber. Laser was directed to the collagen-coated optical substrate in the flow chamber. The photodetector picked up laser light. When the device was on, the whole blood moved inside the flow chamber at a predetermined velocity. The shear rate was ≈1300 s – 1, which is characteristic of arteries with moderately stenosed lumen [6]. While moving inside the flow chamber, blood cells, primarily platelets, interacted with the collagen coating and adhered to it. This caused laser scattering, which increased as more cells adhered to the substrate surface. The scattered laser light picked up by the photodetector was converted to electric potential and measured in millivolts (mV). Thus, higher electric potential at the photodetector output reflected an increase in the degree of cell adhesion to the collagen-coated substrate. Blood circulation in the system and photodetector signal recording were conducted within 15 minutes. The degree of platelet adhesion was determined by the maximum value of the photodetector signal at the end of a 15-minute circulation. A change in the intensity of scattered laser light recorded by the photodetector was registered and processed using L-Graph 2 v.2.35.16 (L–CARD, RF), which allows presenting such changes in a graph. At the second stage of the experiment, mAb to platelet GP1b receptors 10 μg was added in the whole blood sample and 15 minute blood circulation was repeated in a new flow chamber. Measurement results were compared between the patient groups.

Statistical analysis

The quantitative data collected during the study are presented as the means ± standard deviations and the medians and quartiles (25th and 75th percentiles). Statistical hypotheses on the distribution types were tested using the Shapiro-Wilk test. The comparative analysis of the patients data of both groups was performed using the methods of non-parametric statistics: Fisher's exact test and Yates χ^2 test for qualitative variables, the Mann-Whitney U-test for quantitative variables in two independent groups, the Kruskal-Wallis test for quantitative variables in three or more independent groups, and the Wilcoxon test for quantitative variables in two dependent groups. The relationship between the decrease in GPIb mediated platelet adhesion and the presence of premature CAD expressed through the odds ratio was evaluated using logistic regression analysis. The statistical significance was p=0.05. All tests were two-tailed. Statistical analysis was performed using Statistica v. 6.0 (StatSoft Inc., USA) and SPSS Statistics v.17.0 (SPSS Inc., USA).

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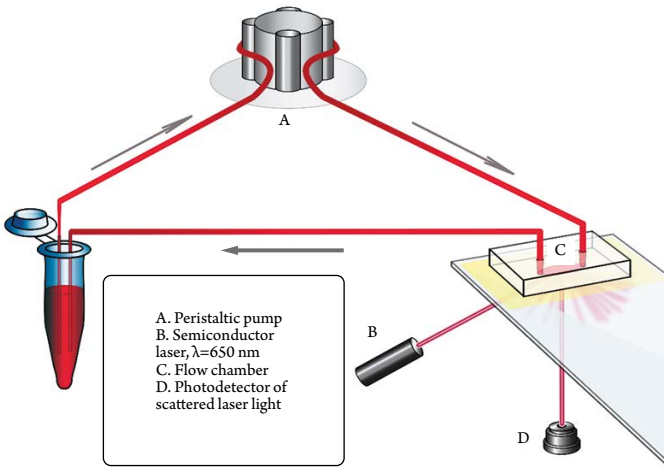
Figure 1. Microfluidic device used to register platelet adhesion kinetics in the controlled flow



Results

Table 1 presents the characteristics of patients with premature CAD and patients of the control group. Patients in both groups did not differ in obesity and family history of CAD. Patients with premature CAD were mainly male, more likely to have diabetes mellitus, arterial hypertension, and hyperlipidemia. In both groups, there were comparable numbers of active smokers and those who stopped smoking more than 6 months before being included in the study. The pack-year indicator was higher in the premature CAD group.

Table 1. Clinical characteristics

Parameter	Premature CAD group (n=58)	Control group (n=33)	p
Age, years	52.6±5.7	46.3±10.8	0.0005
Male/female, n (%)	45 (78) / 13 (22)	13 (40) / 20 (60)	0.0005
Family history of CAD, n (%)	14 (24.1)	7 (21.2)	0.8
Hyperlipidemia, n (%)	54 (93.1)	25 (75.7)	0.02
Smoking, n (%)	33 (56.9)	12 (36.3)	0.08
Active smokers, n (%)	*18 (54.6)*	*5 (41.6)*	*0.1*
Former smokers, n (%)	*15 (45.4)*	*7 (58.4)*	*0.7*
Pack years	*34.2±17.7*	*12.5±9.3*	*0.0007*
Heavy smokers, n (%)	*23 (69.7)*	*2 (16.6)*	*0.0004*
Obesity, n (%)	33 (56.9)	18 (54.5)	0.8
Diabetes mellitus, n (%)	14 (24.1)	1 (3)	0.008
Arterial hypertension, n (%)	54 (93.1)	24 (72.7)	0.01

CAD, coronary artery disease; pack year is a mean number of cigarettes smoked per day multiplied by the number of years of smoking and divided by 20; heavy smoker is an individual who smoked ≥25 pack years.

Patients with premature CAD were more likely to be heavy smokers.

The first clinical manifestations of premature CAD occurred at the mean age of 47 ± 6 years. MI was the first manifestation of CAD in 43% of cases. The incidence of MI as the first manifestation of CAD did not differ in male and female patients. Before being included in the study, 64% of patients underwent coronary artery stenting, and 9% of patients had a history of coronary artery bypass grafting. Hemodynamically significant lesion of the anterior descending artery was detected by CAG in 72% of patients, circumflex artery – 47%, right coronary artery – 55%, main trunk of the left coronary artery – 13%.

The results of the platelet adhesion evaluation according to the administered antiplatelet therapy are presented in Table 2. There was a baseline difference in the degree of adhesion (p=0.04). There were no differences between patients taking acetylsalicylic acid, clopidogrel, dual antiplatelet therapy, and those without therapy, in the degree of adhesion when GPIb platelet receptors were inhibited and in the relative change in adhesion after blocking GPIb receptors compared to the baseline value expressed in %. Thus, the reduction in platelet adhesion, when GPIb receptors were inhibited, was independent of antiplatelet therapy.

The results of platelet adhesion evaluation in patients with premature CAD and patients without CAD are presented in Table 3. The median platelet adhesion value in patients with premature CAD, which was assessed by the maximum increase in the intensity of scattered laser, was 9.1 mV, and after adding mAb to GPIb platelet receptors to the blood, it was 2.5 mV, i.e., 74.8 (55.6; 82.7) % less than without GPIb receptor blocking. In the control group, the median platelet adhesion after the circulation of whole blood without mAb to GPIb platelet receptors was 15.2 mV, and 11.3 mV after inhibiting of GPIb receptors by mAb, i.e., 28.9 (– 9.8; 50.5) % less than without GPIb receptor blocking. In the entire patient population, the median decrease in platelet adhesion after GPIb blocking was 62.8 (52.2; 71.2) %. Blocking of the GPIb receptor interaction with vWF by mAb in patients with premature CAD led to a greater decrease in platelet adhesion compared to patients of the control group (p=0.0001).

The curves of changes in the intensity of scattered laser light depending on the time of blood circulation in the flow chamber in patients with premature CAD and the control group are shown in Figure 2 and Figure 3, respectively.

Logistic regression analysis was used to determine the relationship of the reduction in platelet adhesion following GPIb receptor blocking and traditional risk factors (male sex, age, burdened family history of CAD, diabetes mellitus, hyperlipidemia, arterial hypertension, obesity, and smoking) with the presence of premature CAD. Four independent variables (decreased platelet adhesion after GPIb receptor blocking ≥62.8%, male sex, age, diabetes mellitus) influenced

the quality of the regression logistic model. Other variables had not effect or degraded the model quality, and thus were excluded. The model produced 81.6% of correct predictions (p<0.001). Decreased platelet adhesion after GPIb receptor blocking ≥ 62.8%, adjusted for traditional CAD risk factors, increased the likelihood of having premature CAD (OR=9.84, 95% CI 2.80–34.59; p<0.001).

Discussion

Von Willebrand factor is one of the components of the hemostasis system that has a key role in the platelet adhesion to the subendothelium when the integrity of the endothelial layer is compromised. Exposure of the components of the subendothelial extracellular matrix initiates the vWF attachment to the vascular wall collagen. Binding sites in the vWF A1 domains begin to interact with GPIb platelet receptors, which causes their capture from the bloodstream and adhesion to the artery wall [7–9]. GPIb receptors blocking will disrupt such interaction and thus inhibit adhesion. We hypothesized that disruption of the vWF interaction with GPIb platelet receptors using mAb may differ in patients with premature CAD and patients without CAD. In other words, a contribution of the vWF interaction with GPIb platelet receptors to parietal thrombus formation at the initial stage will be different. The results of this study confirmed our hypothesis.

The vWF is present in the bloodstream in one of two conformations – globular (inactive) and unfolded (active). The vWF conformation depends on the shear rate in the vessels. At a low shear rate, the vWF remains in a globular form hiding its binding sites and, as a result, does not interact with circulating platelets. At a high shear rate, the vWF unfolds and opens its binding sites [6]. Our microfluidic device allows us to control blood velocity in the flow chamber and achieve the shear rate required for the vWF to unfold.

Table 2. Platelet adhesion according to antiplatelet therapy

Antiplatelet therapy	Patients (n)	Baseline adhesion, mV	Adhesion after addition of anti-GPIb mAb, mV	Δ, %
Untreated	5	9.9 (5.9; 13.3)	2.6 (1.4; 5.5)	70.3 (51.7; 82.4)
ASA	11	11.0 (8.3; 15.9)	3.5 (1.7; 10.1)	55.7 (47.9; 84.7)
Clopidogrel	7	5.7 (4.4; 6.4)	1.3 (1.0; 1.8)	72.7 (55.6; 83.4)
ASA + Clopidogrel	35	10.4 (6.0; 18.3)	2.0 (1.6; 3.4)	76.6 (66.2; 82.8)
p	–	0.04	0.14	0.7

ASA, acetylsalicylic acid; mV, millivolt; mAb, monoclonal antibodies; GPIb, glycoprotein Ib; Δ – relative change in platelet adhesion after blocking GPIb receptors by mAb compared with the baseline value, expressed in %; p – comparison of three or more independent groups (Kruskal-Wallis test). Daily doses of ASA and clopidogrel were 100 mg and 75 mg, respectively. Blood samples were collected in 5 patients at admission before ordering antiplatelet therapy.

In the vast majority of studies devoted to the effect of vWF on the occurrence and the course of CAD, plasma levels were measured using ELISA assays [10–13]. In a prospective study, 1411 male patients without CAD were divided by tertiles depending on the vWF levels. After a maximum follow-up of 16 years, upper tertile patients, after the adjustment for common CAD risk factors, faced a higher risk of CAD than lower tertile patients (OR 1.53, 95% CI 1.10–2.12) [14]. However, according to a large ARIC study, which included

Figure 2. Curves of changes in the intensity of scattered laser light before and after mAb blocking of GPIb platelet receptors in patients with premature CAD

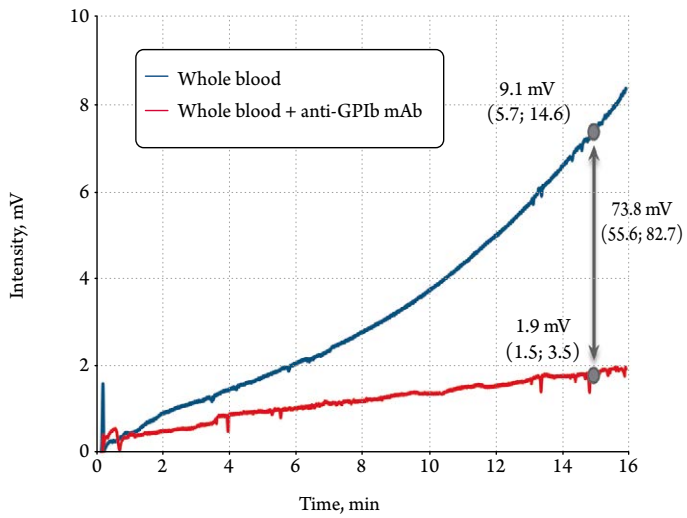
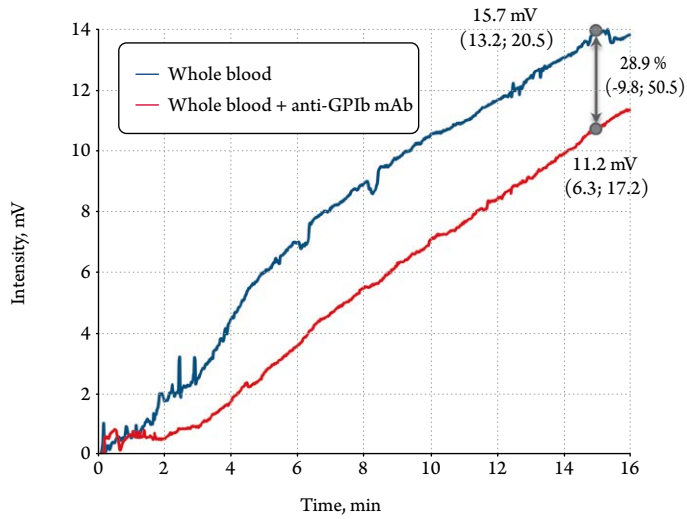


Figure 3. Curves of changes in the intensity of scattered laser light before and after mAb blocking of GPIb platelet receptors in patients of the control group



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14,477 subjects of 45–64 years old, elevated vWF levels can be considered as a risk factor for CAD, however, adding the elevated VWF levels to traditional risk factors has little effect on the prediction of the disease onset [15]. Many studies found a direct relationship between the levels of vWF and the occurrence of adverse cardiovascular events in patients with CAD when compared with the control [16–18]. The prospective ECAT study included 3,043 patients with angina pectoris. The duration of the study was 2 years. It showed that patients with a history of MI or sudden cardiac death had higher baseline vWF levels in the blood. Patients were divided by quantiles depending on the vWF levels. The risk of adverse cardiovascular events was 1.85 times higher in upper quantile patients than in lower quantile patients [19]. It should be noted, however, that measuring the vWF levels in the blood plasma is limited, since it does not allow findings the ratio of functionally active and inactive forms of the vWF. The vWF activity is assessed using a ristocetin-cofactor test, which determines its ability to bind to GPIb platelets when affected by antibiotic ristocetin. This test allows identifying severe vWF dysfunction. However, the interaction of GPIb platelets with the vWF occurs in the ristocetin-cofactor test under the effect of a chemical agent, which does not allow evaluating the physiological function of the vWF [20]. Many factors are engaged in the clotting process. The development of new diagnostic methods to identify violations of individual links of this complex chain, including those aimed at assessing the functional activity of the vWF under as authentic natural conditions as possible, can contribute to the development of new drugs.

Table 3. Platelet adhesion in patients with premature CAD and patients of the control group

Groups	Baseline, mV	anti-GPIb mAb, mV	Δ, %	p
Premature CAD group (n=58)	9.1 (5.7; 14.6)	1.9 (1.5; 3.5)	74.8 (55.6; 82.7)	<0.0001
Control group (n=33)	15.7 (13.2; 20.5)	11.2 (6.3; 17.2)	28.9 (–9.8; 50.5)	0.03

CAD, coronary artery disease; mV, millivolt; mAb, monoclonal antibodies; GPIb, glycoprotein Ib; Δ – relative change in platelet adhesion after blocking GPIb receptors by mAb compared with the baseline value, expressed in %; p – comparison of the baseline value with the value after adding anti-GPIb mAb (Kruskal–Wallis test).

Limitations

The study was limited by a small size of the patient groups.

Conclusion

Inhibition of the interaction of GPIb receptors with the vWF at elevated shear rates in patients with premature CAD leads to a greater decrease in platelet adhesion compared with patients without CAD. This suggests that excessive interaction of the vWF with platelets may be involved in the pathogenesis of premature CAD.

No conflict of interest is reported.

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REFERENCES

1. Gulati R, Behfar A, Narula J, Kanwar A, Lerman A, Cooper L et al. Acute Myocardial Infarction in Young Individuals. Mayo Clinic Proceedings. 2020;95(1):136–56. DOI: 10.1016/j.mayocp.2019.05.001
2. Zeitouni M, Clare RM, Chiswell K, Abdulrahim J, Shah N, Pagidipati NP et al. Risk Factor Burden and Long-Term Prognosis of Patients With Premature Coronary Artery Disease. Journal of the American Heart Association. 2020;9(24):e017712. DOI: 10.1161/JAHA.120.017712
3. Neeland IJ, Patel RS, Eshtehardi P, Dhawan S, McDaniel MC, Rab ST et al. Coronary angiographic scoring systems: An evaluation of their equivalence and validity. American Heart Journal. 2012;164(4):547-552.e1. DOI: 10.1016/j.ahj.2012.07.007
4. Gabbasov ZA, Avtaeva YN, Melnikov IS, Okhota SD, Caprnda M, Mozos I et al. Kinetics of platelet adhesion to a fibrinogen-coated surface in whole blood under flow conditions. Journal of Clinical Laboratory Analysis. 2021;35(9):e23939. DOI: 10.1002/jcla.23939
5. Avtaeva Yu.N., Mel'nikov I.S., Gabbasov Z.A. Real-Time Recording of Platelet Adhesion to Fibrinogen-Coated Surface under Flow Conditions. Bulletin of Experimental Biology and Medicine. 2018;165(1):157–60. [Russian: Автаева Ю.И., Мельников И.С., Габбасов З.А. Регистрация в реальном времени адгезии тромбоцитов на иммобилизованном на оптической подложке фибриногеновом покрытии в условиях потока. Клеточные технологии в биологии и медицине. 2018; 1:48-52]. DOI: 10.1007/s10517-018-4119-5
6. Rana A, Westein E, Niego B, Hagemeyer CE. Shear-Dependent Platelet Aggregation: Mechanisms and Therapeutic Opportunities. Frontiers in Cardiovascular Medicine. 2019;6:141. DOI: 10.3389/fcvm.2019.00141
7. Lancellotti S, Sacco M, Basso M, Cristofaro RD. Mechanochemistry of von Willebrand factor. Biomolecular Concepts. 2019;10(1):194–208. DOI: 10.1515/bmc-2019-0022
8. Stuklov N.I., Kobelevskaya N.V., Polikarpova T.S., Chistyakova A.V., Ogurtsov P.P. Physiology and pathology of hemostasis. -M.: GEOTAR-Media;2016. - 112 p. [Russian: Стуклов Н.И., Кобелевская Н.В., Поликарпова Т.С., Чистякова А.В., Огурцов П.П. Физиология и патология гемостаза. - М.: ГЭОТАР-Медиа, 2016. - 112с]. ISBN 978-5-9704-3625-7
9. Avdonin P.P., Tsvetaeva N.V., Goncharov N.V., Rybakova E.Yu., Trufanov S.K., Citrina A.A. et al. Von Willebrand factor in norm and pathology. Membrane and Cell Biology. 2021;38(4):237–56. [Russian: Авдонин П.П., Цветаева Н.В., Гончаров Н.В., Рыбакова Е.Ю., Труфанов С.К., Цитрина А.А. и др. Фактор Виллебранда в норме и при патологии. Биологические мембраны. 2021;38(4):237-56]. DOI: 10.31857/S0233475521040034
10. Rutten B, Maseri A, Cianflone D, Laricchia A, Cristell N, Durante A et al. Plasma levels of active Von Willebrand factor are in-

creased in patients with first ST-segment elevation myocardial infarction: A multicenter and multiethnic study. European Heart Journal: Acute Cardiovascular Care. 2015;4(1):64–74. DOI: 10.1177/2048872614534388
11. Li Y, Li L, Dong F, Guo L, Hou Y, Hu H et al. Plasma von Willebrand factor level is transiently elevated in a rat model of acute myocardial infarction. Experimental and Therapeutic Medicine. 2015;10(5):1743–9. DOI: 10.3892/etm.2015.2721
12. Chion CKNK, Doggen CJM, Crawley JTB, Lane DA, Rosendaal FR. ADAMTS13 and von Willebrand factor and the risk of myocardial infarction in men. Blood. 2007;109(5):1998–2000. DOI: 10.1182/blood-2006-07-038166
13. Willeit P, Thompson A, Aspelund T, Rumley A, Eiriksdottir G, Lowe G et al. Hemostatic Factors and Risk of Coronary Heart Disease in General Populations: New Prospective Study and Updated Meta-Analyses. PLoS ONE. 2013;8(2):e55175. DOI: 10.1371/journal.pone.0055175
14. Whincup P, Danesh J, Walker M, Lennon L, Thomson A, Appleby P et al. von Willebrand factor and coronary heart disease. Prospective study and meta-analysis. European Heart Journal. 2002;23(22):1764–70. DOI: 10.1053/euhj.2001.3237
15. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective Study of Hemostatic Factors and Incidence of Coronary Heart Disease: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 1997;96(4):1102–8. DOI: 10.1161/01.CIR.96.4.1102
16. Jansson JH, Nilsson TK, Johnson O. von Willebrand factor in plasma: a novel risk factor for recurrent myocardial infarction and death. Heart. 1991;66(5):351–5. DOI: 10.1136/hrt.66.5.351
17. Rumley A, Lowe GD, Sweetnam PM, Yarnell JW, Ford RP. Factor VIII, von Willebrand factor and the risk of major ischaemic heart disease in the Caerphilly Heart Study. British Journal of Haematology. 1999;105(1):110–6. PMID: 10233372
18. Ray KK, Morrow DA, Gibson CM, Murphy S, Antman EM, Braunwald E. Predictors of the rise in vWF after ST elevation myocardial infarction: implications for treatment strategies and clinical outcome. European Heart Journal. 2005;26(5):440–6. DOI: 10.1093/eurheartj/ehi104
19. Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW. Hemostatic Factors and the Risk of Myocardial Infarction or Sudden Death in Patients with Angina Pectoris. New England Journal of Medicine. 1995;332(10):635–41. DOI: 10.1056/NEJM199503093321003
20. Budde U, Pieconka A, Will K, Schneppenheim R. Laboratory Testing for von Willebrand Disease: Contribution of Multimer Analysis to Diagnosis and Classification. Seminars in Thrombosis and Hemostasis. 2006;32(5):514–21. DOI: 10.1055/s-2006-947866

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