OVERALL HEMOSTASIS POTENTIAL OF BLOOD PLASMA AND ITS CONNECTION TO MOLECULAR MARKERS OF THE HEMOSTASIS SYSTEM IN PATIENTS WITH STENOSIS OF CORONARY ARTERY

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The correlation relationships between hemostatic potential parameters and concentrations of molecular markers of the hemostasis system: soluble fibrin (sf), D-dimer (DD), fibrinogen (Fg) and protein C (PC) in patients with stenosis of coronary artery 6 months after coronary angioplasty have been investigated. It was found three directions of changes in the state of the patients hemostasis system: an increasing in fibrinolytic activity (C) ~18% of patients; an increasing in coagulative activity (B) ~31% of patients; and maintaining of the balance between coagulation and fibrinolysis (A) ~51% of patients. In patients with signs of stenosis without angina pectoris, a strong Pearson correlation was shown between the half-life of the clot and the overall hemostatic potential (OHP) (r = 0.75, P << 0.05), a moderate relationship between concentrations of sf and D-dimer (r = 0.67, P <0.05), almost complete connection between coagulation potential (CP) and OHP (r = 0.975, P << 0,05) and strong connection between CP and fibrinolytic potential (FP) (r = 0.80, P << 0.05). In patients with signs of stable angina pectoris, almost complete connection was found between the concentration of sf and D-dimer (r = 0.981, P << 0.05), CP and OHP (r = 0.979, P << 0.05) and a strong connection between CP and FP (r = 0.846, P << 0.05). Possible functional mechanisms of connection between these parameters are discussed.

K e y w o r d s: stenosis, angina pectoris, hemostasis potential of plasma, soluble fibrin, D dimer, fibrinogen.

Currently the most common cause of death in the world among non-communicable disease is ischemic heart disease, which in 2019 accounted for 16% of all deaths in the world. At the same time, the increase in mortality due to this disease is growing. Thus, in the period from 2019 to 2000, mortality from it increased by more than 2 million cases and reached a total of 8.9 million cases per year. Vascular stenosis and angina are among the most dangerous ischemic diseases [1, 2]. Ischemic heart disease is caused by atherosclerosis and stenosis of the heart coronary arteries. Stenosis occurs due to narrowing in some places of the cardiac vessels. The most common cause of narrowing is atherosclerotic plaque, which blocks part of the lumen of the vessel and creates an obstacle to blood flow. Another dangerous ischemic disease is angina, the main cause of which is also atherosclerotic lesion of the coronary arteries and blood vessels of the heart of patients, which in addition to narrowing the lumen of the coronary artery is accompanied by inflammation in the endothelial wall of blood vessels [3, 4]. In both cases, stenosis of the coronary arteries causes "wall shear stress" in the vessels [5, 6], which leads to the unfolding of Willebrand factor in the bloodstream, activation and aggregation of platelets,

what release of procoagulant nanoparticles into the bloodstream [7, 8], which cause activation of the coagulation system with the formation of thrombin and soluble fibrin [9, 10], that ultimately contributes to the formation of arterial thrombi. In the coronary system, the inflammatory process stimulates the procoagulant activity of endothelial cells [11], which enhances the formation of arterial thrombi.

Our task was to determine the values of hemostatic potential and concentration of molecular markers of blood plasma hemostasis system state of patients with coronary heart disease 6 months after coronary angioplasty to identify predictors of late restenosis and angina pectoris, and to haracterize the presence and strength of the correlation between these parameters.

Materials and Methods

The study examined a group of patients of 93 persons of both sexes with stenotic lesion of the coronary artery. The diagnosis was confirmed by coronary angiography. The age of patients ranged from 42 to 76 years (mean age was 56.4 years, in patients with stenosis – 56.4 years and in patients with unstable angina pectoris – 57 years). The study included patients who underwent coronary artery angioplasty with the insertion of one eluting-stent (Taxus, Boston Scientific). All patients received dual antiplatelet therapy according to the protocol, which consisted of 75 mg of clopidogrel and 100 mg of aspirin per day for 6 months, antihyperlipidemic therapy (rosuvastatin 10-20 mg), β-adrenoblocker (bisoprolol 5 mg) and ACE inhibitor (ramipril 5-10 mg). Before coronary angiography, patients underwent general clinical examinations (general blood test, creatinine, level of transaminases, total protein, bilirubin). Six months after coronagraphy (according to the unified clinical protocol of primary, secondary (specialized), tertiary (highly specialized) medical care from 02.03.2016 No 152 with changes from 23.09.2016 No 994) 44 patients were diagnosed with stable angina pectoris. At the moment of blood sampling for the study of the hemostasis system, patients did not receive anticoagulant and fibrinolytic therapy.

Permission from the Ethics Commission of Pirogov's Vinnytsia National Medical University (protocol No 12 dated 16.11.2016), and informed consent of each patient were obtained to conduct research related to blood.

Blood samples from donors and patients with signs of stenosis without angina pectoris (hereinaf-ter stenosis) and with signs of stable angina pectoris (hereinafter angina) were withdrawn in 3.8% sodium citrate (1 part sodium citrate and 9 parts of blood, pH 7.4). Plasma was separated from blood cells within 1 hour after blood collection by centrifugation at 1000 g for 20 min. Plasma aliquots were stored at -20°C.

APTT (activated partial thromboplastin time) reagent from Renam (RF), recombinant tissue plasminogen activator (t-PA) from Boehringer Ingelheim (Germany) were used in experiments. The hemostatic potential of blood plasma was determined by turbidimetric method with recording the scattering of light by a fibrin clot at 405 nm on a microreader Multiscan (Finland). The clot was formed in the microplate wells, to which were successively added 0.05 M HEPES buffer, pH 7.4, containing 0.15 M NaCl, 70 μl of blood plasma, t-PA to a final concentration of 75 IU/ml, (or without t-PA addition) and APTT reagent. The plasma coagulation process was initiated by the addition of 25 mm $CaCl_2$. The final volume of the reaction mixture was 300 μl [12].

Overall hemostasis potential (OHP) was characterized by the area under the turbidity curve of the clot from the moment of initiation of plasma coagulation to the moment of complete destruction of the clot in the presence of t-PA. Coagulation potential (CP) – the area under the curve of clot formation from the moment of initiation of plasma coagulation to the moment of complete dissolution of the clot in the absence of t-PA. Fibrinolytic potential (FP) is the difference between the values of CP and OHP. All values were expressed in units of optical density multiplied by time in seconds (o.u.·s) [12, 13].

The individual stages of the process of clot formation and hydrolysis were characterized by the following parameters: τ (s) – lag period, which shows the rate of activation of the internal path of the coagulation cascade, thrombin formation, fibrinogen activation into fibrin and protofibril formation; α (V_1) o.u./s – tg of the angle of inclination of the maximum rate of growth of the clot turbidity, which shows the rate of lateral association of protofibrils and the formation of fibrils; H (o.u.) – the magnitude of the final turbidity of the clot, which is determined by the thickness of the fibrils of the clot; L (s) is the half-life of the clot, which depends on the concentration of plasminogen, t-PA and the structure of the clot; β ($-V_2$) – o.u. /s – tg of the angle of inclination of the maximum rate of falling of turbidity of a clot which depends on concentration of plasmin and

plasmin inhibitors (α2-antiplasmin, PAI-1) in blood plasma [13].

The concentration of fibrinogen, soluble fibrin and D dimer in blood plasma was determined with enzyme-linked immunosorbent assay using test systems developed at Palladin Institute of Biochemistry of NAS of Ukraine [14, 15], and the level of protein C – using a test system from Renam (RF). Statistical processing of the results was performed using a standard statistical program in "Excel". The mean values of the parameters and their standard deviations were determined. The significance of the difference between the mean values of the two groups was determined by the Student's test and Pearson's pairwise correlation analysis (the Pearson correlation coefficient – r) was performed.

Results and Discussion

General characteristics of the hemostasis system in patients with stenosis and angina. To determine the parameters of the hemostatic potential of blood plasma of each patients, turbidity curves of blood coagulation processes initiated by APTT reagent in the absence and presence of t-PA were obtained. Typical experimental curves for patients with stenosis are presented in Fig. 1, *A*, *B*, *C*, and for patients with angina - in Fig. 2, *A*, *B*, *C*. Analysis of the array of turbidity curves of both types of disease allowed to divide them into three groups according to the shape of the turbidity curve obtained in the presence of t-PA, namely: (A) – curves which maximum turbidity coincides with such curves in the absence of t-PA; (B) – curves which maximum turbidity exceeds that of turbidity curves in the absence of t-PA and (C) – curves with a maximum turbidity that is less than that of curves in the absence of t-PA, Fig. 1 and Fig. 2. Quantitatively, patients were divided into groups:

	A	B	C	Total
Stenosis	22	18	9	49
	(44.9%)	(36.7%)	(18.4%)	(100%)
Angina	23	13	8	44
	(52.3%)	(29.5%)	(18.2%)	(100%)

The obtained data indicate that in 44.9 and 52.3% of patients with stenosis and angina, respectively, the formation and hydrolysis of the fibrin clot in blood plasma does not differ in shape from that observed in normal blood plasma of donors, Fig. 1, *A* and Fig. 2, *A*. 55.1% with stenosis and 47.7% of patients with angina have atypical forms of turbi-
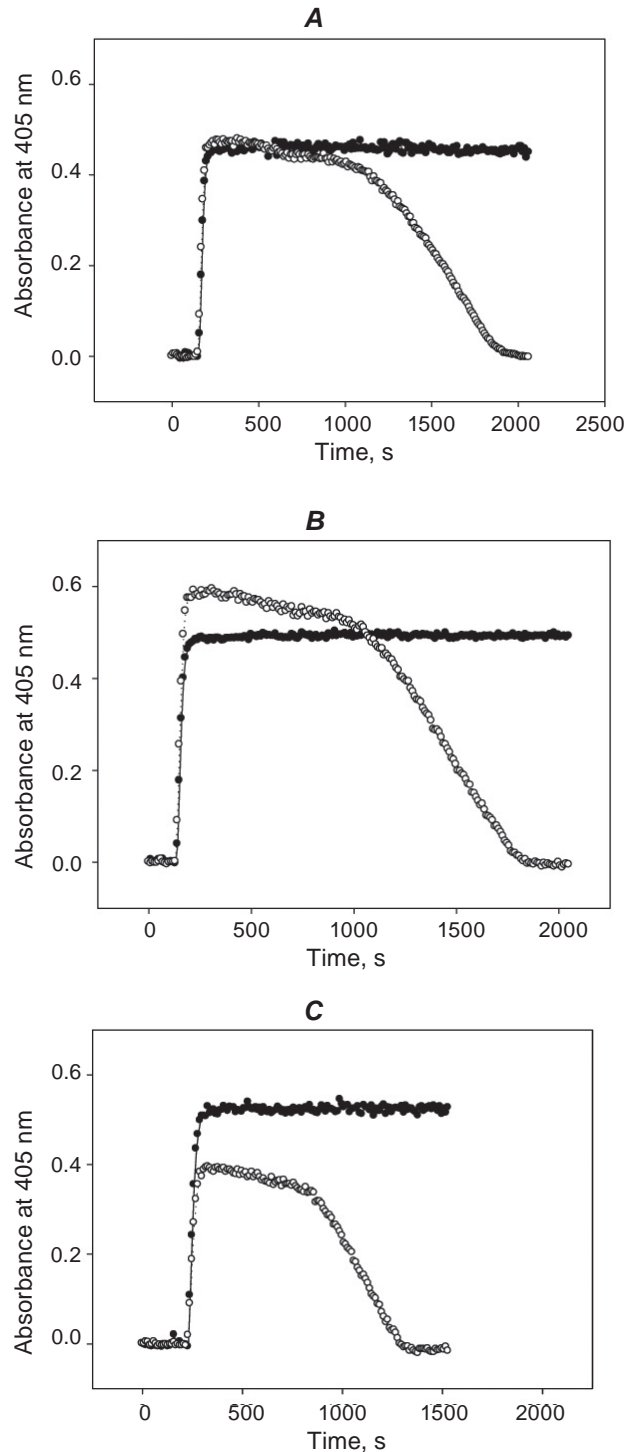

*Fig. 1. Typical turbidity curves of blood plasma coagulation initiated by APTT reagent in the presence (light circle) and absence of t-PA (dark circle), in patients with stenosis. **A** – turbidity curves of blood plasma coagulation are similar to those of donor blood plasma; **B** – turbidity curves of blood plasma coagulation with increased coagulation activity; **C** – turbidity curves of blood plasma coagulation with increased fibrinolytic activity*

dimetric curves obtained in the presence of t-PA. 36.7% and 29.5% of patients with stenosis and angina show an increased level of turbidity of the clot, which indicates increased activity of the clotting system Fig. 1, *B* and Fig. 2, B, and 18.4% and 18.2% of patients with stenosis and angina, respectively, show a reduced level of turbidity of the clot Fig. 1, *C* and Fig. 2, *C*, which can be explained by the increased level of activity of the fibrinolytic system of blood plasma in these groups of patients.

The values of the parameters of turbidity curves characterizing the activation of the coagulation system (τ, α, H, CP) in group A of patients with stenosis and donors do not differ in the absence of t-PA, Table 1. Among the molecular markers there is a ~2 times increased level of soluble fibrin and reduced by 2 times the normal concentration of D-dimer, which indicates a general increased coagulation and reduced fibrinolytic activity in the hemostasis system of this group of patients. In group B, in the absence of t-PA, the values of the parameters of the turbidity curve coincide with those of the donors, except for the important ratio of CP/FP – 5.96 ± 1.32 and 3.89 ± 0.62, respectively, which indicates increased activity of the coagulation system [12, 13]. This observation is confirmed by an increase in the level of soluble fibrin in 5.62 times relatively to the donor level. In the C group of patients where ratio of CP/FP was 3.12 ± 0.62, a decrease in the level of soluble fibrin, and an increase in the concentration of D-dimer indicated on increased fibrinolytic activity in this group plasmas comparing to others groups of cardiac stenosis patients.

In patients with stenosis activation of the blood plasma coagulation system is observed in all groups, but this does not cause activation of the fibrinolysis system. It is likely that the polymerization of soluble fibrin, which may occur in groups A and B, is effectively inhibited by fibrinogen (antithrombin I), the concentration of which is ~1.6 times higher the concentration of Fg in donors, but is at the upper limit of normal concentration in humans, Table 1. Obviously, soluble fibrin does not form fibrin microclots and does not stimulate plasmin formation. Prolongation of the half-life of the clot in all groups of patients with stenosis in the presence of t-PA indicates an increase in the concentration of fibrinolysis inhibitors, apparently PAI-I, in blood plasma. However, the hemostatic potential ratio of CP/FP, equal to 5.96 ± 1.32, indicates a shift in the balance between the processes of coagulation and fibrinolysis in the
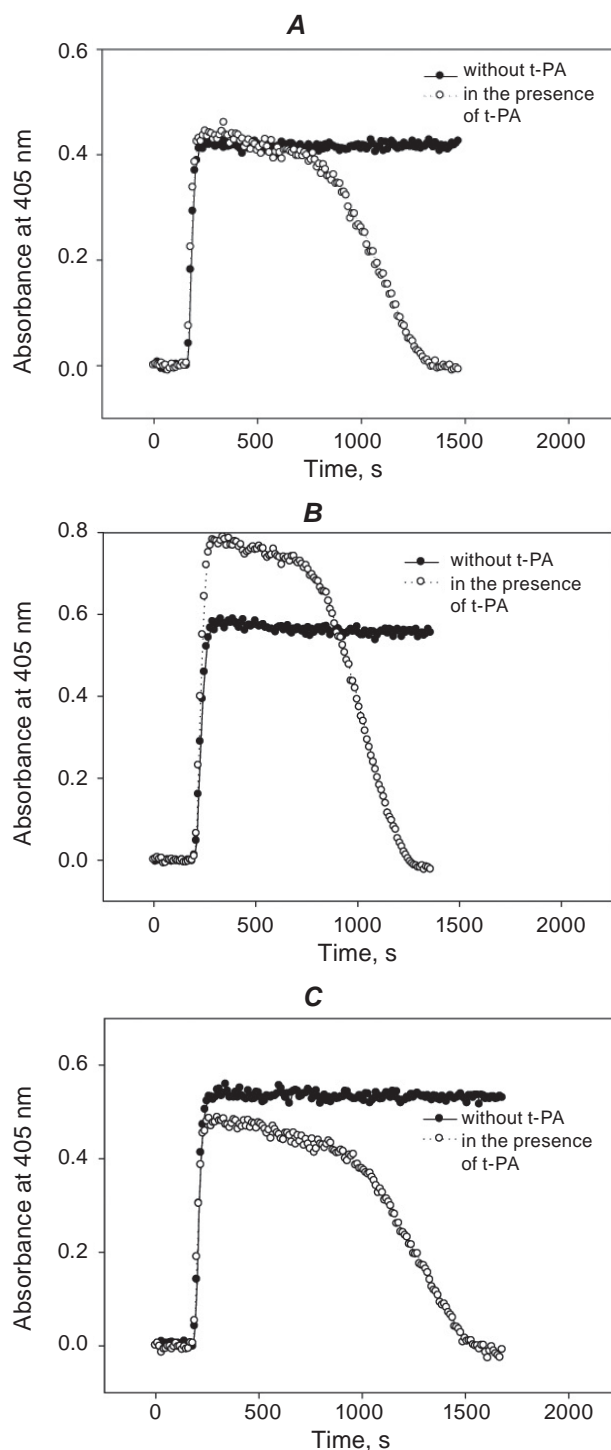

*Fig. 2. Typical turbidity curves of blood plasma coagulation initiated by APTT reagent in the presence and absence of t-PA, in patients with angina. **A** – turbidity curves of blood plasma coagulation are similar to those of donor blood plasma; **B** – turbidity curves of blood plasma coagulation with increased coagulation activity; **C** – turbidity curves of blood plasma coagulation with increased fibrinolytic activity*

T a b l e 1. Values of parameters of hemostatic potential, soluble fibrin (sf), D dimer (DD), fibrinogen (Fg) and protein C (PC) in plasma of patients with stenosis (n = 49). Patients are divided into three subgroups depending on the shape of the turbidity curve: A form – 22 patients, B form – 18 patients, C form C – 9 patients

Parameters	In the absence of t-PA				In the presence of t-PA	
	τ, s	α, o. u./s	H, o.u.	CP, o. u.·s	OHP, o. u.·s	FP, o. u.·s
Donors	140±23	0,0085±0.0012	0.381±0.052	345±17*	257.5±15.0	88.5±11*
All patients	166±36	0.0088±0.0024	0.372±0.106	399±245	307±185	92±75*
A Form	134±26	0.008±0.0017	0.338±0.078	421±243	249±187*	100±71
B Form	156±32	0.0091±0.0024	0.373±0.105	487±280	386±227*	83±57*
C Form	169±38	0.0110±0.0023	0.455±0.130	507±208*	342±146	167±74*
	n.d.	n.d.	n.d.	P = 0.019	P = 0.025	P = 0.004, P < 0.0001, n.d., P < 0.009
	In the presence of t-PA					
Parameters	CP/FP	τ, s	α, o. u./s	H, o. u.	L, s	H_o, o. u.
Donors	3.89±0,53	145±23	0.0092±0.00012	0.375±0.043	893±31	0.332±0.153
All patients	4.65±1.44*	167±31	0.0085±0.0022	0.378±0.105	1137±235	0.470±0.227
A Form	4.21±0.74*	174±26	0.0080±0.0019	0.340±0.078*	1104±228	0.475±0.223
B Form	5.96±1.32*	157±33	0.0091±0.0024	0.411±0.115*	1211±256	0.480±0.175
C Form	3.05±0.62*	169±38	0.0092±0.0023	0.402±0.127	1069±184	0.480±0.185
	P = 0.001, P = 0.001, P = 0.001	n.d.	n.d.	P = 0.023	n.d.	n.d.
	In the presence of t-PA		Molecular markers of the hemostasis system			
Parameters	β, o. u./s		Sf, µg/ml	DD, ng/ml	Fg, µg/ml	PC, %
Donors	-0.00038±0.00011		2.6±0.67*	62 ±16*	2.80±0.36*	100
All patients	-0.00027±0.00044		12.8±31.2	48.6±50.9	4.26±1.47*	94.4±18.6
A Form	-0.00023±0.00040		14.4±32.4	30.6±14.5*	4.27±1.38	93.2±19.9
B Form	-0.00031±0.00044		14.7±37.78	61.2±58.8	4.19±1.66	97.4±19.2
C Form	-0.00028±0.00045		5.29±3.26*	67.00±7.77*	4.40±1.42*	91.3±14.7
	n.d.		P = 0.015	P = 0.001, P = 0.001	P = 0.002, P = 0.002	n.d.

n.d. – There is not a statistically significant difference between the mean values of each of the two groups; *statistically significant difference between the mean values of indicated groups

direction of coagulation only for patients in group B. In group A, the balance between coagulation and fibrinolysis (4.21 ± 0.74), is maintained. In group C, the equilibrium is shifted toward fibrinolysis and the ratio is 3.12 ± 0.62, respectively (donors – 3.89 ± 0.53 [13]). It should be noted that the difference in the phenomenology of turbidity curves in patients of groups B and C reflects the opposite directions of

changes in the state of hemostasis of blood plasma in these groups of patients.

Thus, the shape of the turbidity curve indicates in the case of patients with stenosis (group A) an increased level of activation of the blood coagulation system and slight shifts in the balance between CP and FP (4.21 ± 0.74) towards coagulation. In the case of patients of group B there is an activation of

the blood plasma coagulation system and a significant shift of the balance between CP and FP in the direction of activation of the coagulation system (5.96 ± 1.32). In case C, there is a significant shift in the balance between the processes of coagulation and fibrinolysis in the direction of activation of the latter (3.05 ± 0.65).

The values of the parameters of turbidity curves that characterize the activation of the coagulation system (τ, α, H, CP) in group A of patients with angina and donors are given in Table. 2. The rate of fibrinogen activation and protofibril formation slows down 1.29-1.38 times in patients of group A and group B. In the presence of t-PA, the rate of protofibril formation and the rate of clot half-life are decreased by 1.24 times. However, the rate of dissolution of the clot structure (β) in the plasma of patients of all groups increases ~ 2 times. The ratio of CP/FP in the groups of patients with angina is in group A – 4.65 ± 1.51, in group B – 7.05 ± 2.79, in group C – 2.90 ± 0.32, which indicates a shift in equilibrium in the direction of blood plasma clotting in group B and in fibrinolysis – in group C. It should be noted a lower concentration of soluble fibrin in the blood plasma of all groups of patients with angina compared with those in patients with stenosis and a lower concentration of D-dimer in group C. There are also little differences in the plasma concentrations of Fg and PC in patients of the corresponding groups with stenosis and angina. It should also be noted the increased level of initial turbidity in the blood plasma of all groups in stenosis by 1.44 and angina 1.59 times higher those of the blood plasma of donors, which in units of optical density is 0.138 and 0.202 at 405 nm, respectively. It can be assumed that these values indicate the presence in the plasma of patients nanoparticles, which are released into the blood by platelets, monocytes, endothelial cells [9] at the sites in the affected coronary artery with stenosis or angina and may indicate the degree of vascular damage. The rate of lateral association of protofibrils (α) for patients with stenosis and angina does not differ. However, the rate of destruction of the structure of the clot (β) in the plasma of patients with stenosis is much higher than in the plasma of patients with angina, respectively (stenosis/angina): in group A – $0.63 \cdot 10^{-3}/0.23 \cdot 10^{-3} = 2.7$; in group B – $0.58 \cdot 10^{-3}/0.31 \cdot 10^{-3} = 1.9$; in group C – $0.51 \cdot 10^{-3}/0.28 \cdot 10^{-3} = 1.6$ times. It has previously been shown that in a pure protein system, the half-life of a clot depends on the plasmin concentration [16]. Since the half-life of the clot in plasma of patients with ste-

nosis and angina did not differ, the concentration of plasmin formed in both cases should be the same, indicating the same rate of plasminogen activation in both clots. Inhibition of the process of clot destruction by plasmin indicates a higher concentration of plasmin inhibitors, possibly covalently incorporated by factor XIIIa into the structure of the clot [17], and those (α2-antiplasmin, PAI-1) presented in the blood plasma of patients with stenosis. This may be due to the presence of wall shear stress, which increases the population of activated platelets, which together with procoagulant nanoparticles release plasmin inhibitors into the bloodstream [18].

Thus, changes in the parameters of turbidity curves of formation and dissolution of fibrin clot in blood plasma, hemostatic potentials and concentrations of molecular markers of hemostasis system state in patients with stenosis and angina are similar at these diseases and indicate three directions of changes in the state of the hemostasis system: an increase in fibrinolysis (C) ~18% of patients; an increase in coagulation (B) ~31% of patients; and maintaining a balance between coagulation and fibrinolysis (A) ~51% of patients.

Analysis of the values of the turbidity curves parameters and the concentrations of molecular markers of the state of the hemostasis system showed similar directions of their changes in patients with both diseases. In this regard, it was important to investigate the presence and strength of correlations between functionally related parameters of the hemostasis system in the entire group and subgroups of patients separately, which was estimated by the value of Pearson's correlation coefficient [13]. The studied parameters were divided into three groups: the first one included the parameters of the turbodity curve of formation and dissolution of the fibrin clot (τ, α, β, H); to the second – coagulation, overall and fibrinolytic potentials (CP, OHP and FP), and the third - the concentration of molecular markers of the hemostasis system (Fg, PC, D dimer, sf).

In case of stenosis in the first group of parameters of the turbidity curve no significant correlation was found between τ, α, H and β parameters in the absence and presence of t-PA, both within the group and with concentrations of molecular markers – Fg, PC, D dimer and sf. In group B patients, a strong correlation was found in the pairs L-OHP and (L- τ) –OHP with $r = 0.75$ and $r = 0.76$, respectively, and $P << 0.05$ for each of the two pairs. In group C of patients with stenosis for the third group of parameters revealed only one moderately

T a b l e 2. Values of parameters of hemostatic potential, soluble fibrin (sf), D dimer (DD), fibrinogen (Fg) and protein C (PC) in plasma of patients with angina pectoris (n = 44). Patients are divided into three subgroups depending on the shape of the turbidity curve: A form – 23 patients, B form – 13 patients, C form – 8 patients

	In the absence of t-PA				In the presence of t-PA	
Parameters	τ, s	α, o. u./s	H, o.u.	CP, o. u.·s	OHP, o. u.·s	FP, o. u.·s
Donors	40±23*	0.0085±0.0012	0.381±0.052	345±17	257,5±15	88.5±11.0*
All patients	184±39*	0.0079±0.0027	0.347±0.110	374±161	307±185	90±75
A Form	180±33*	0.008±0.0017	0.352±0.079	388±162	309±129	92±49
B Form	188±128	0.0080±0.0020	0.367±0.156	369±147	384±276	59±31*
C Form	195±65*	0.0074±0.0039	0.334±0.135	344±191	225±128	119±64*
	$P = 0.008$, $P < 0.001$, $P = 0.002$	n.d.	n.d.	n.d.	n.d.	$P = 0.003$, $P = 0.008$

	In the presence of t-PA					
Parameters	CP/FP	τ, s	α, o. u./s	H, o. u.	L, s	H$_o$, o. u.
Donors	3.89±0.53*	145±23*	0.0092±0.00012	0.375±0.043	893±31*	0.332±0.153*
All patients	4.01±2.28	185±39	0.0073±0.0027	0.358±0.120	1137±235	0.534±0.218*
A Form	4.65±1.51*	180±33*	0.0082±0.0025	0.363±0.109	1095±234*	0.529±0.260*
B Form	7.05±2.79*	189±29*	0.0087±0.0034	0.419±0.171	1111±288*	0.501±0.161*
C Form	2.90±0.32*	195±65*	0.0068±0.0036	0.302±0.116	977±157	0.528±0.182*
	$P < 0.001$, $P = 0.022$, $P < 0.001$	$P = 0.002$, $P < 0.001$, $P = 0.028$	n.d.	n.d.	$P = 0.006$, $P = 0.021$	$P = 0.020$, $P = 0.027$, $P = 0.016$, $P = 0.017$

	In the presence of t-PA	Molecular markers of the hemostasis system			
Parameters	β, o. u./s	Sf, µg/ml	DD, ng/ml	Fg, µg/ml	PC, %
Donors	-0.00038±0.00011	2.60±0.67	62 ±16*	2.80 ±0.36*	100
All patients	-0.00057±0.00027	7.06±13.8	60.5±131.3	4.42±1.37*	93.3±16.2
A Form	-0.00063±0.00040	6.96±11.8	85.1±131.3	4.50±1.39*	92.0±19.1
B Form	-0.00058±0.00039	9.2±20.7	40.0±31.2*	4.64±1.35*	97.6±19.1
C Form	-0.00051±0.000056	3.46±1.89	31.6±26.5*	4.07±0.61*	89.3±12.8
	n.d.	n.d.	$P = 0.046$, $P = 0.005$	$P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$	n.d.

n.d. – There is not a statistically significant difference between the mean values of the two groups; *statistically significant difference between mean values of the indicated groups

strong correlation between the concentration of sf and D dimer with $r = 0.67$ and $P < 0.05$. The latter result confirms our assumption about the involvement of blood cells and the transepithelial transport mechanism of removal of soluble fibrin from the bloodstream through the blood-tissue barrier in cardiac arteries stenosis [19, 20].

The data on the presence of a strong positive correlation between the half-life of the clot and the value of the overall hemostasis potential in patients of group B with stenosis are also noteworthy. This result indicates the dependence of activation and functioning of the fibrinolytic system in patients with increased activity of the blood plasma coagu-

lation system on the value of OHP, which is determined by the protein composition, size and structure of fibrin clot, the formation and lysis of which in vitro is initiated by APTT reagent.

At the same time, a moderate (but quantitatively on the border of strong -0.67 and -0.7 strong) negative correlation between the length of the lag period and the half-life of the clot in patients of group B indicates the molecular processes that determine the protein the composition, structure, magnitude and time (rate) of the half-lysis of the clot, namely: the rate of activation of the internal coagulation pathway and thrombin formation, the rate of transformation of Fg in fibrin monomer and in parallel the same activation of factor XIII, the rate of self-assembly of fibrin in protofibrils, inclusion of γ'-fibrin into protofibrils and crosslinking of fibrin via γ-γ chains and covalent incorporation of α2-AP and PAI-1 fibrinolysis inhibitors into protofibrils [21, 22]. This result shows that the higher the rate of these molecular processes, the more stable the structure of the fibrin clot to lysis by the fibrinolytic system of blood plasma [23]. The negative correlation between the rate of fibrin clot formation in blood plasma and the rate of its lysis *in vitro* by the fibrinolytic system confirms the kinetic mechanism of regulation of hemostatic balance in blood plasma of patients with coronary arteries stenosis [23].

In angina, as in stenosis, in the first group of parameters of the turbidity curve no correlations were found within the group between the values of τ, α, H parameters in the absence and presence of t-PA. In a pair of parameters ΔH - FP was found a strong positive correlation with $r = 0.71$ and $P < 0.05$ in group C, which was absent in other groups of patients. An increase in the value of the parameter ΔH (the difference between the H turbidity curve obtained in the absence and presence of t-PA) indicates a decrease in the value of OHP, which is probably due to inflammation in the coronary system, leukocyte activation and release of proteolytic enzymes into the bloodstream. and among them is elastase, which cleaves αC regions from Fg, forming an X-fragment that is able to accelerate Pg activation and lysis of the clot [24, 25]. For the L– OHP pair in patient groups A and B, there was a strong correlation with $r = 0.72$ and 0.88, respectively, with the same $P << 0.05$. Between the parameters - the time of dissolution of the clot (L- τ) and the concentration of DD and sf was shown a weak and moderate relationship, respectively, with $r = 0.46$ and $P << 0.05$

and $r = 0.59$ and $P < 0.05$ for patients in group A. As in the case of stenosis, in angina there is a strong correlation between the parameters (L-τ) -OHP in the blood plasma of patients in all groups A, B and C with r and P, respectively, 0.7 and $P << 0.05$, 0.89 and $P << 0.05$ and 0.72 and $P = 0.046$.

In contrast to patients with coronary arteries stenosis, where only in group C there is a moderate correlation between sf and DD, in all patients with angina there is a correlation between sf and DD in group A almost complete with $r = 0.981$ and $P << 0.05$, in group B strong with $r = 0.86$ and $P << 0.05$ and in group C strong correlation with $r = 0.75$ and $P << 0.05$, Fig. 3. Since a strong correlation between the concentration of the main molecular markers - sf and DD, which directly reflects the balance between coagulation and fibrinolytic components of the hemostasis system in patients with angina *in vivo* at the time of blood sampling is observed in all patient groups, then we can assume that in the vessels of the coronary circulatory system localized a powerful system of fibrinolysis, which effectively hydrolyzes the formed sf.

However, another mechanism for balance regulation between sf and DD concentrations is possible. Given that fibrin desA, which forms sf, has a plasma half-life of 2 hours [26], and DD - 9-16 hours [27, 28], and that the molecular weight of fibrin desA is 1.8 times greater than this DD, and that all D dimer is formed by hydrolysis of sf, to form 0.085 µg/ml concentration of DD must be cleaved 0.17 µg/ml sf or 2.4% of its concentration to maintain a balanced level of concentrations that we observe in patients with angina, Table. 2. In this case, 97.6% of sf should be removed by blood cells and vascular endothelium in the circulatory system [23, 29].

The study of correlations between hemostatic potentials in the *in vitro* system showed that for all patients with angina and stenosis there is an almost complete relationship between the values of coagulation and total hemostatic potential, Fig. 4 and Fig. 5. At the same time its value does not change essentially depending on group of patients. Thus, for all patients with angina $r = 0.996$ with $P << 0.05$, and for groups A, B, and C, respectively, $r = 0.964$ with $P << 0.05$, $r = 0.991$ with $P << 0.05$ and $r = 0.996$ with $P << 0.05$. For all patients with stenosis $r = 0.979$ with $P << 0.05$ and for groups A, B, and C, respectively, $r = 0.988$ with $P << 0.05$, $r = 0.996$ with $P << 0.05$ and $r = 0.973$ with $P << 0.05$. In CP - FP parameters pair, a strong relationship is maintained
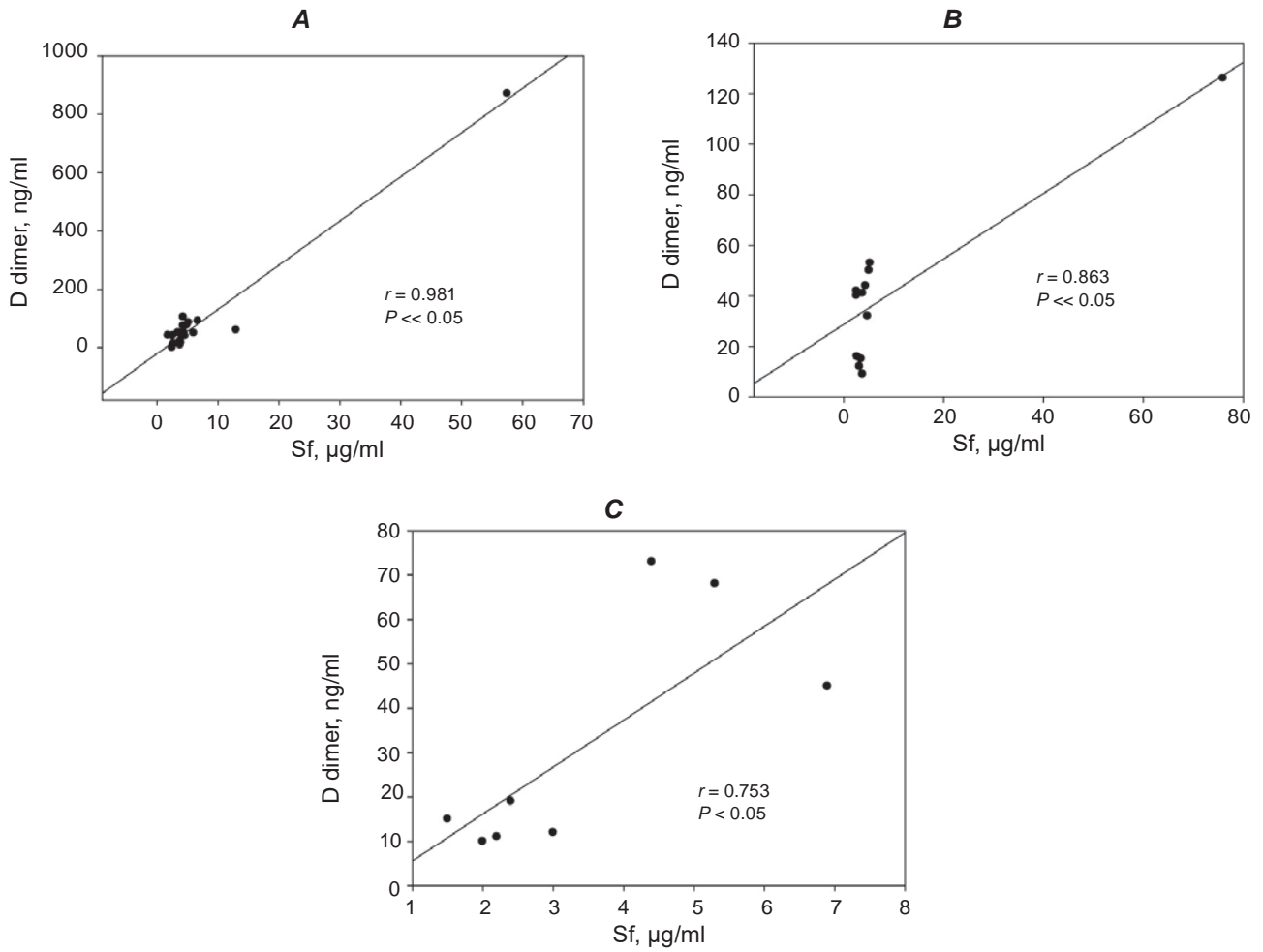
*Fig. 3. Plot of Pearson correlation relationship between values of sf and D dimer concentrations for angina patients of group A (n = 23) (**A**), group B (n = 13) (**B**) and group C (n = 8) (**C**)*
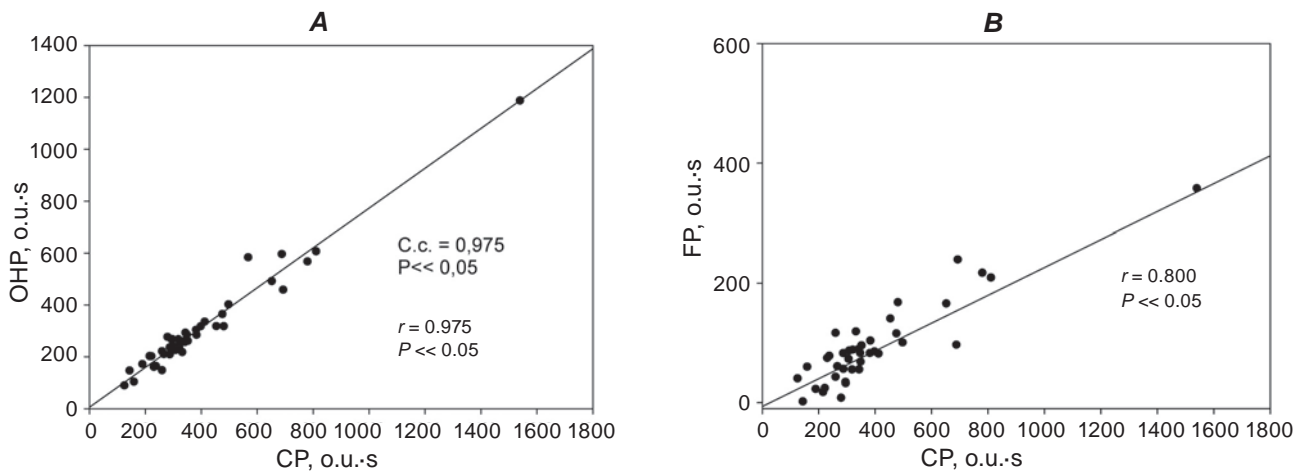

*Fig. 4. Plot of Pearson correlation relationship between values of coagulation and overall hemostasis potential (**A**) and coagulation and fibrinolytic potential (**B**) of blood plasma of all patients with angina*
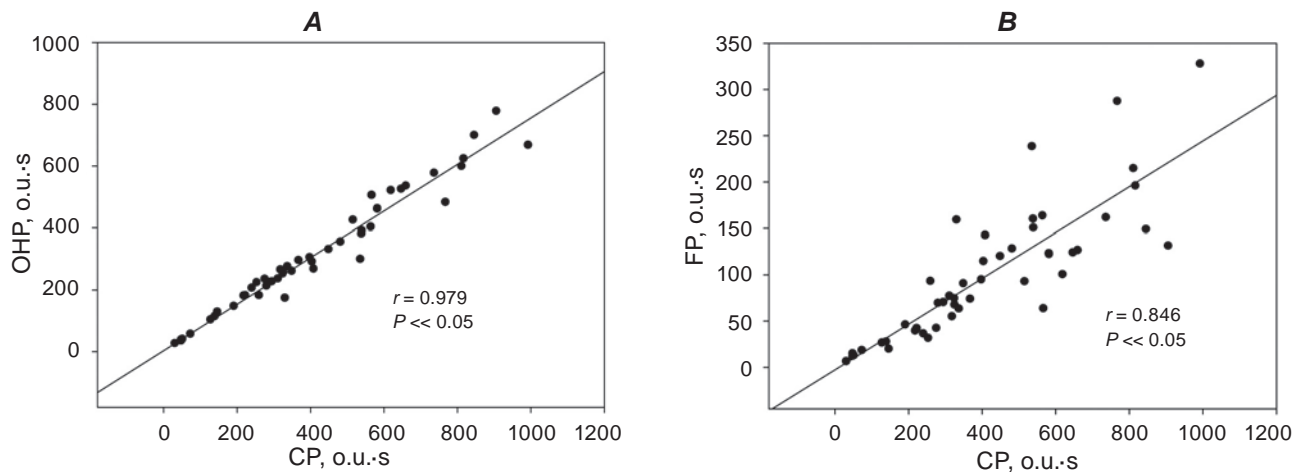
*Fig. 5. Plot of Pearson correlation relationship between coagulation and overall hemostatic potential (**A**) and coagulation and fibrinolytic potential (**B**) of blood plasma of all patients with coronary stenosis*

for all patients with angina and stenosis, respectively, $r = 0.80$ with $P \ll 0.05$ and $r = 0.846$ with $P \ll 0.05$. For all patients with stenosis for the pair OHP - FP there is a strong relationship $r = 0.77$ with $P \ll 0.05$, and for patients with angina the strength of the relationship decreases to moderate – $r = 0.67$ with $P \ll 0.05$.

The obtained data confirm the conclusion that the general directions of changes of coagulation and fibrinolytic parameters of the state of the hemostasis system of blood plasma of patients with stenosis and angina have similar tendencies.

ЗАГАЛЬНИЙ ГЕМОСТАТИЧНИЙ ПОТЕНЦІАЛ ПЛАЗМИ КРОВІ ТА ЙОГО ЗВ'ЯЗОК ІЗ МОЛЕКУЛЯРНИМИ МАРКЕРАМИ СИСТЕМИ ГЕМОСТАЗУ В ПАЦІЄНТІВ ЗІ СТЕНОЗОМ КОРОНАРНОЇ АРТЕРІЇ

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Вивчали зв'язок між показниками гемостатичного потенціалу та концентраціями молекулярних маркерів системи гемостазу: розчинного фібрину (sf), D-димеру (DD), фібриногену (Fg) та протеїну С (PC) в пацієнтів зі стенозом коронарної артерії через 6 місяців після стентування. У хворих було виявлено три напрями зміни стану системи гемостазу: збільшення фібринолітичної активності у ~18% пацієнтів; збільшення коагуляційної активності (B) у ~31% пацієнтів; підтримка балансу між згортанням і фібринолізом (A) в ~51% пацієнтів. У пацієнтів зі стенозом без ознак стенокардії продемонстровано сильну кореляцію за Пірсоном між часом напівлізису згустку та загальним гемостатичним потенціалом (ЗГП) $r = 0,75$, $P \ll 0,05$), помірна залежність між концентраціями sf – D-димер ($r = 0,67$, $P < 0,05$), майже повний зв'язок між потенціалом згортання (ЗП) і ЗГП ($r = 0,975$, $P \ll 0,05$) та сильний зв'язок між ЗП і фібринолітичним потенціалом (ФП) ($r = 0,80$, $P \ll 0,05$). У пацієнтів із ознаками стабільної стенокардії виявлено майже повний зв'язок між концентрацією sf та D-димеру ($r = 0,981$, $P \ll 0,05$), ЗП і ЗГП ($r = 0,979$, $P \ll 0,05$) та сильний зв'язок між ЗП та ФП ($r = 0,846$, $P \ll 0,05$). Обговорюються можливі функціональні механізми зв'язку між цими параметрами.

К л ю ч о в і с л о в а: стеноз коронарної артерії, гемостатичний потенціал плазми, розчинний фібрин, D-димер, фібриноген.

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