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Features of hemostasis in patients with non-ST-elevation myocardial infarction

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Abstract

Background: Coronary thrombosis is the key pathogenic mechanism of acute heart attack, including non-ST segment elevation (NSTEMI). Given that, the detection of reliable markers of hemostasis disorders is important in the process of optimizing the diagnosis of NSTEMI.
Material and methods: The study was conducted on 54 patients with NSTEMI (average age 69.7±1.5 years). In 60% of cases, 3-vessel disease was noted; 56% of patients had ejection fraction >50%, and Killip class I of heart failure was revealed in 78% of patients. With the help of the STA-Liatest (France) equipment, the blood tests determined the following hemostasis markers: fibrin monomers (FM), thrombotic complex activity of factors II, VII and X. Additional markers like Procoag, the coagulation indicator dependent on circulating phospholipids or SPA, D-dimers, as well as factors C, S and antithrombin III were appreciated. The values of these markers determined by the same method in 20 healthy persons (control group) were used as normal values.
Results: Circulating level of FM on admission was increased twice, while the values of Procoag and SPA were significantly decreased by 35.3% compared to the control. Factors C, S and antithrombin III were 54-80% of the control value range, and D-dimers were within the permissible values. In the acute phase of the heart attack, a deterioration of hemostasis indicators was noted, excepting the D-dimers. The levels of FM determined 24 and 72 hours after revascularization were consistently increased (up to 3.8 times) compared to the control, while Procoag and SPA decreased by 54-57%. Further reduction of factors C, S and antithrombin III accounted for 42-54% of normal indicators. After 5 days, an improvement in hemostasis markers was observed, but a significant difference still remained comparing to the control group.
Conclusions: The hemostasis particularities discovered in patients with NSTEMI indicate the features of an activated prothrombotic status, and FM could be an important diagnostic marker of NSTEMI, due to its most significant deviation from the normal value (>100%). It can reliably reflect the thrombin level, which triggers the last enzymatic phase of thrombus formation.
Key words: NSTEMI, hemostasis disorders.

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Introduction

Impairment of hemostasis manifested by increased prothrombotic activity is common in vascular accidents (acute myocardial infarction and cerebrovascular accident) which show the highest cardiovascular mortality rate [1]. The formation of thrombus in the lumen of arteries and arterioles is classically confirmed pathophysiologically as an imbalance between the coagulation and anticoagulant systems in combination with the failure of the fibrinolysis system, which leads to a decrease in arterial blood flow and triggers the ischemic cell necrosis. From pathogenic point of view, the prothrombotic phenomenon can be initiated and sustained by endothelial damage and inflammation, platelet activation and oxidative stress, blood rheological disorders and genetic polymorphism correlating with the expression of hemostasis regulation factors, etc. [2].

Given the certain relationship between coronary atherosclerosis and acute myocardial infarction with ST-segment elevation (STEMI) or without (NSTEMI), intracoronary thrombosis is, according to the Guidelines of the European Society of Cardiology, the determining cause of the infarction, leading to total occlusion of the coronary artery in STEMI or subtotal in NSTEMI [3]. In NSTEMI, thrombosis of the subendocardial arteries (arterioles <200 μm in diameter) can lead to the development of subendocardial myocardial infarction even when the subepicardial artery (culprit artery) is less than 75% occluded. The severity of thrombotic conditions and dysfunction of the coronary microcirculatory system correlates with the prognosis of NSTEMI and explains the risk of infarction even when an average-to-minimal gradient of transmural coronary perfusion is maintained. However, in the European Society of Cardiology Guidelines for the management of STEMI, hemostasis parameters are not included in the diagnostic and prognostic biomarker algorithm, and the long-term periprocedural and postinfarction treatment (up to 12 months) is based on antithrombotic drugs (anticoagulant and/or antiplatelet therapy).

Hemostasis represents a complex and multi-hierarchical system of homeostasis, and prothrombotic events derive from intrinsic and/or extrinsic triggered cascade of reactions

resulting in the formation of respective tenases linked to the endothelial injury and activation of oxidative stress, inflammatory response and blood rheological disorders.

Thus, the assessment of key indicators of hemostasis can strengthen important landmarks for the pathophysiological detection of prothrombotic preconditioning and the prediction of the risk of NSTEMI as well as the identification of diagnostic and prognostic predictors.

In this aspect, the aim of the study was: comprehensive assessment of hemostasis markers in patients with NSTEMI. The main objectives of the study:

1. Evaluation of the functional feasibility of the main components of hemostasis (coagulation, anticoagulation, fibrinolysis) in order to identify the inherent pathogenic mechanisms, which is an argument in favor of the pathogenetic targeted treatment.

2. Evaluation of hemostasis indicators not only at admission, but also during different periods of the acute phase of MI without ST segment elevation (24 hours, 72 hours, 5 days), which has the greatest impact on the survival of a patient undergoing angioplasty.

Material and methods

The study was conducted on a group of 54 patients with NSTEMI who underwent primary coronary angioplasty in the interventional cardiology laboratory within the institutional project "*Evaluation of instrumental and biochemical markers in the management of patients with acute myocardial infarction without ST segment elevation, as well as in the assessment of the degree of coronary microvascular damage*". The general characteristics of the patients included in the study are presented in table 1. The indices detected during coronary angiogram are presented in table 2.

Table 1. Clinico-demographic indicators of patien

Indices	N	%	M±m
Age, years			69.7±1.5
Men	35	65	
Women	19	35	
Hypertension	50	92.6	
Dyslipidemia	46	86	
Diabetes	18	34	
Smoking	19	36	
Atrial fibrillation	13	24	
History of stroke	8	15	
Heart failure (HF), Killip Class I	42	78	
Killip Class II	10	19	
Killip Class III	2	3	
Ejection fraction (EF) >50%	30	56	
EF 40-49%	11	21	
EF <40%	5	10	
EF ≤ 35%	8	13	
Glomerular filtration rate, ml/min			60.7±3.26
GRACE score			129±6.4
TIMI score			4.75±0.2

Table 2. Indicators of coronary artery injury in patients with NSTEMI

Number of injured coronary arteries (stenosis >50%)			
1-VD	2-VD	3-VD	LMS
11 (21%)	9 (17%)	32 (60%)	2 (4%)
culprit coronary artery			
LAD 30 (56%)		LCX 16 (30%)	RCA 8 (15%)

The main blood values of hemostasis in all NSTEMI patients were assessed using the STA-Liatest laboratory equipment (France) at admission and 24h, 72h and 5 days after revascularization.

Regarding coagulant activity:

- Factor-dependent coagulation index (II, VII and X) or prothrombotic complex activity index (ICP), estimated in seconds (sec).
- Circulating procoagulant phospholipids (IFP) coagulation index, estimated in sec.
- Fibrin monomers (FM) as an indicator of plasma thrombin activity, estimated in mg/ml.
- Regarding anticoagulant activity:
- Protein C, estimated as % from acceptable normal reference value (4 mg/L).
- Protein S, estimated as % from acceptable normal reference value (35 mg/L).
- Antithrombin III (AT III), estimated in % from accepted reference value (< 250 ng/ mL).
- Regarding fibrinolytic activity:
- D-dimers, estimated in mg/ml.

All the above indices of hemostasis were also appreciated in 20 apparently healthy people (control group), using the same laboratory equipment.

The group of patients with NSTEMI assessed for hemostasis did not include people with raised procoagulant status due to autoimmune diseases (systemic lupus, rheumatoid arthritis), deep vein thrombosis, pregnancy, oral hormonal drugs, contraceptives, cancer, etc. All patients with NSTEMI who underwent coronary angioplasty received pre- and post-procedural therapy for up to 12 months including antiplatelet agents and, if necessary, anticoagulants in accordance with the Guidelines for STEMI and PCI [3, 4].

In order to exclude antiphospholipid syndrome, which has a convincing prothrombotic effect, the value of antiphospholipid antibody (lupus anticoagulant) was determined using the Dilute Russell Viper Venom Time (DRVVT) blood test [5]. There was no significant difference between the values of the DRVVT parameter between groups (tab. 3).

Table 3. Value of the DRVVT index (sec)

The control lot M±SD	NSTEMI patient cohort M±SD	P
39.6 ± 4.8	41.7 ± 6.5	>0.05

Evaluation of the laboratory analysis did not reveal any indicators that could affect hemostasis in patients with

NSTEMI, the hematocrit level being at 38.6±1.7%, and the platelet count 246±14 x 103/μl. The degree of inflammatory response upon admission was determined by the level of highly sensitive protein C (hsCRP) in the blood serum, equal to an average of 11.4 mg/l, and the average value of ESR (erythrocyte sedimentation rate) of 22±3 mm/hour.

Statistical processing of the obtained digital material included the determination of the mean (M) and standard deviation (SD). When comparing indicators between groups (control and patients with NSTEMI), the discrepancy was considered significant at $p<0.05$.

Results

The admission values of hemostasis indices are presented in table 4.

Table 4. Hemostasis parameters of patients with NSTEMI before revascularization

Parameters and their reference ranges according to the method	Control	NSTEMI	P vs control
ICP, sec (60-80)	72.3±9.1	46.8±6.6	<0.01
IFP, sec (70-130)	83.6±9.4	61.5±7.2	<0.001
MF, mg/ml (0.1-6.0)	4.7±0.8	9.5±1.3	<0.001
Protein C, % (70-130)	85.4±10	59.4±7.6	<0.001
Protein S, % (60-170)	88.1±9.9	47.7±6.4	<0.001
Antithrombin III, % (80-120)	92.6±11	73.4±6.3	<0.05
D-dimers, mg/ml (0-0.5)	0.29±0.05	0.41±0.08	<0.001

Analysis of the results indicates an accentuated prothrombotic pattern at the admission of patients with NSTEMI, given a significant decrease in the values of ICP and IFP by 35.3% and 26.4%, respectively, compared to the control value. The reduction in clotting time, dependent on the prothrombin complex and phospholipids, was accompanied by a two-fold increase in the level of circulating fibrin monomers (9.5±1.3 vs. 4.7±0.8 mg/ml), which reflects a high level of thrombin. In this context, it should be noted that the explored anticoagulant system indices are imposed by significantly reduced admission values. Thus, the value of protein C and protein S was estimated to be below the control value by 30.5% and 45.9%, respectively. The antithrombin III value was significantly reduced by 19.7% (74.4±6.3 vs 92.6±11%).

The blood concentration of D-dimers fell within the reference range of the imminent index of the used laboratory method, but significantly rose above the value of the control group by 42% (0.41±0.08 vs 0.29±0.05 mg/ml).

Therefore, the impairment of the functional capacity of the anticoagulant system is in an intelligible relationship with the procoagulant augmentation.

Following 24 hours from the moment of revascularization of patients with NSTEMI, the procoagulant activity becomes more pronounced (tab. 5).

Table 5. Hemostasis parameters of NSTEMI patients 24 hours after myocardial revascularization

Parameters and their reference ranges according to the method	Control lot	NSTEMI	Deviations vs admission	P vs control
ICP, sec (60-80)	72.3±9.1	34.2±5.3	-26.9%*	<0.001
IFP, sec (70-130)	83.6±9.4	50.4±6.5	-18.1%*	<0.001
MF, mg/ml (0.1-6.0)	4.7±0.8	12.6±1.8	+33%*	<0.001
Protein C, % (70-130)	85.4±10	51.7±6.5	-13%	<0.001
Protein S, % (60-170)	88.1±9.9	41.4±5.9	-13.2%	<0.001
Antithrombin III, % (80-120)	92.6±11	59.7±6.1	-18.7%*	<0.05
D-dimers, mg/ml (0-0.5)	0.29±0.05	0.44±0.07	+8%	<0.001

Note: * – significant discrepancy vs admission level.

At the distance of 24 hours after revascularization, there was a significant decrease in ICP and FPI values compared to the admission level by 26.9% and 18.1%, respectively. The result of this decline was the increase and the rebound of the indices compared to the control value, which became equal to 52.7% and 39.7%, respectively. On the background of the reduction of the coagulation time dependent on the prothrombotic complex, the blood content increased by 33%, a fact that determined an incremental gap of the index compared to the control by 2.68 fold.

Although, after 24h, the procoagulant activity was in a notable rise, the value of the anticoagulant proteins C and S decreased insignificantly with an average rate of 13%. However, it is worth mentioning the significant depreciation of AT III by 18.7%.

The circulating level of D-dimers increased insignificantly (by 8%) and remained at the maximum level of the index references accepted by the estimation method.

The changes in hemostasis indices at 72 hours after revascularization are important, when the inflammatory response reaches its peak intensity, taking into account the maximum expression of M1 pro- inflammatory macrophages (tab. 6).

Table 6. Hemostasis parameters of patients with NSTEMI, 72 hours after myocardial revascularization

Parameters and their reference ranges according to the method	Control lot	NSTEMI	Deviations vs admission	P vs control
ICP, sec (60-80)	72.3±9.1	31.3±4.1	-33.1%*	<0.001
IFP, sec (70-130)	83.6±9.4	37.8±5.3	-38.5%*	<0.001
MF, mg/ml (0.1-6.0)	4.7±0.8	17.9±2.1	+89%*	<0.001
Protein C, % (70-130)	85.4±10	50.2±6.3	-15.5%*	<0.001
Protein S, % (60-170)	88.1±9.9	40.1±5.1	-15.4%*	<0.001
Antithrombin III, % (80-120)	92.6±11	53.8±5.9	-26.7%*	<0.001
D-dimers, mg/ml (0-0.5)	0.29±0.05	0.50±0.06	+22%*	<0.001

Note: * – significant discrepancy vs admission level.

This post-infarction period excels with the maximum reduction of indices reflecting procoagulant activity compared to the admission level: ICP by 33.1% and, in particular, IFP by 38.5%. It is important to emphasize that the coagulation time dependent on the procoagulant phospholipids, decreased from the imminent 24h index, more than the ICP rebound: 25 vs 8.4%. The circulating level of MF increased by 42% and exceeded the control value by 89%.

Another important feature of hemostasis dynamics in patients with NSTEMI at 72h after angioplasty compared to the values at 24h is the insignificant decline in the level of proteins C and S, which was within the limits of 2.9-3.1%. AT III decreased during this period by 9.9% and reached an underlying level compared to the control at the rate of 26.7%.

The average blood content of D-dimers increased by 22% compared to the admission level, but does not exceed the index reference range accepted by the estimation method.

At a distance of 5 days after revascularization, the analysis of the obtained results indicates an attenuation of the prothrombotic activity (tab. 7).

Table 7. Hemostasis parameters of NSTEMI patients 5 days after myocardial revascularization

Parameters and their reference ranges according to the method	Control lot	NSTEMI	Deviations vs admission	P vs control
ICP, sec (60-80)	72.3±9.1	40.5±5.8	-13.4%	<0.001
IFP, sec (70-130)	83.6±9.4	51.4±6.7	-16.4%*	<0.001
MF, mg/ml (0.1-6.0)	4.7±0.8	12.0±1.7	+27%*	<0.001
Protein C, % (70-130)	85.4±10	52.7±6.1	-11.3%	<0.001
Protein S, % (60-170)	88.1±9.9	42.4±5.3	-10.5%	<0.001
Antithrombin III, % (80-120)	92.6±11	56.4±6.2	-23.2%*	<0.001
D-dimers, mg/ml (0-0.5)	0.29±0.05	0.44±0.08	+8%	<0.001

Note: * – significant discrepancy vs admission level.

Thus, the main indices reflecting the procoagulant activity, ICP and IFP, demonstrate an increase of 30% and 36%, respectively, compared to their minimum level recorded after 72 hours. However, their values remain below the admission level by 13.4-16.4%. It should be noted, that in relation to the control indices, ICP and IFP have a significant rebound of 44% and 38.5%, respectively.

The blood content of fibrin monomers decreased to an average rate similar to the decline of ICP and IFP, by 33% from their maximum level during the 72-hour period, but remained significantly higher than the index admission value by 27%.

Protein C and protein S values were insignificantly elevated at 72 hours to 5 days, approaching admission levels with a small difference, but the discrepancy versus control remained consistent at rates between 38.3% and 52%.

The increase in antithrombin III was 5%, and the difference from admission and control remained significant at 23.2% and 39.1%, respectively.

The blood concentration of D-dimers decreased by 8% compared to the maximum level attested at the distance of 72 hours and reached the admission level, consequently maintaining within the range of normal references accepted by the method.

Discussion

The study revealed important changes in hemostasis in patients with NSTEMI, and can strengthen the conclusive pathophysiological landmarks regarding its evolution. On the other hand, the revealed changes can serve as diagnosis predictor, prognosis and optimization of the patient's post-infarction treatment. Hemostasis parameters were determined in 54 patients with NSTEMI using laboratory equipment STA-Liatest (France) at admission, as well as in the acute phase of myocardial infarction. In order to detect the mechanisms which endanger hemostasis, there were evaluated procoagulant markers (ICP, IFP and FM), anticoagulant markers (protein C, protein S and AT III), and D-dimers as a marker of both fibrinolytic enzyme activity and presence of intravascular thrombi, degraded by proteolysis. It should be noted that the group of patients with NSTEMI did not include people with prothrombotic preconditioning, such as deep vein thrombosis, autoimmune diseases (e.g. systemic lupus, rheumatoid arthritis), pregnancy, cancer, long post-traumatic immobilization and/or post- surgery, acute inflammatory process, administration of anticoagulant remedies, affecting blood rheology.

Estimating the connection between the early key events of the post-infarction evolution and the character of hemostasis, it is important to elucidate its contribution as a NSTEMI trigger on the background of subendocardial arteriole thrombosis. Meanwhile, it states the remodeling of the myocardium, starting already in the first hours after the onset of necrosis and involving prothrombotic factors, such as endothelial damage due to angioplasty, activation of oxidative stress and inflammation. In this aspect, hemostasis indices in the post-infarction period were determined at the interval of 24 hours (maximum infiltration of the necrotic area with neutrophils), 72 hours (the period of maximum expression of M1 macrophages) and 5 days, the period of macrophage repolarization (macrophage differentiation M1 in anti-inflammatory macrophages, M2).

The obtained results showed that, upon admission, the coagulation time dependent on the prothrombotic complex (II, VII, X), ICP and dependent on circulating procoagulant phospholipids, IFP was significantly reduced by up to 35.3%. Given the increased blood coagulation in patients with NSTEMI, a double raise in the circulating level of fibrin monomers was also observed. Importantly, the value of ICP and FM is indispensable for thrombin content, which results from the proteolytic cleavage of prothrombin under the action of tenases (intrinsic and extrinsic) and thrombokinase. Once formed, thrombin cleaves plasma fibrinogen into fibrin monomers, stating its direct relation to thrombin concentration [6-8]. Although both tenases finally converge

in a common path of thrombin formation under the action of factors Va and Xa, in atherosclerosis and coronary injuries, extrinsic tenase (tissue factor + factor VIIa) may have a notable role regarding prothrombotic activity, given the endothelial injury. Tissue factor is also a trigger of the inflammatory response, which in turn may increase coronary endothelial injury, resulting in excessive growth factor release.

It is noteworthy that at 24h and 72h from the onset of NSTEMI, marked by neutrophil infiltration and M1 macrophage activation, respectively, the inflammatory response is maximum, and the 33.1% decrease in ICP during these intervals in this study was logically associated with an increase in MF levels in blood up to 89%. Namely, in the 72-hour postinfarction period, recognized as the peak of the inflammatory reaction, the ICP value was minimal, and the MF value was maximal. Thus, there is a close pathophysiological relationship between ICP and FM, and changes in these parameters in patients with non-ST elevation MI, similar to those observed in this study, reflect the activation of the coagulation cascade in both ways and can be diagnostic predictors of NSTEMI.

In addition, the change in the IFP index is in clear contiguity with the change in ICP and correlates with FM. The circulating procoagulant phospholipid-dependent clotting time reduced on admission in NSTEMI patients decreases exponentially at 24 hours and further at 72 hours. Circulating procoagulant phospholipids represent a factor facilitating the formation of tenases by assembling clotting factors in the presence of phosphatidylserine expressed by activated platelets, apoptotic bodies during apoptosis of endotheliocytes, or endothelial exosomes appreciated rather like extracellular vesicles. The formation and activation of external tenase, which is also reflected in the ICP index, occurs much faster in the presence of negatively charged phospholipids [9, 10]. Elevation of circulating procoagulant phospholipids is a direct repercussion of the endothelial injury potentiated by the inflammatory response and oxidative stress in the period between 24-72 hours. In particular, during this period, IFP was observed to decrease by up to 38.5% compared to the admission value, and compared to the control index, the rebound was 54.8%. Similar to the ICP, the decline in the value of IFP index correlated with the increase in the quantitative level of fibrin monomers. Moreover, at a distance of 5 days after revascularization, during the period of attenuation of the inflammatory response caused by expression of M2 anti-inflammatory macrophages, their improvement was noted at similar levels.

The increase of FM blood levels in patients with NSTEMI has been noted in other studies, and an increase similar to the one in this study is reported by M. Arthamin et al. (2019) who applied the ROC curve to demonstrate that at the marker value of 8.2 mg/ml, its specificity and sensitivity towards NSTEMI is 87-90%, which is close to the value of 18 ng/L obtained for troponins (85-91%) [11].

In the present research, the average circulating level of FM at admission was 9.5 mg/ml, which establishes the predictive value of the marker regarding the diagnosis of NSTEMI. Moreover, it is intelligible to assume that FM can show an increasing dynamic up to the moment of the necrotic injury of cardiomyocytes manifested by troponins elevation, also stated by other authors [12-14]. At this point, it is conceptually and practically important to note that, in patients with NSTEMI, the circulating level of FM correlates directly with the level of troponin elevation above the 99th percentile, as well as with the risk of MACE at a distance of 6-12 months.

Thus, there is no doubt that the assessment of the factors that have a definite contribution to the fibrinogen cleavage process under the proteolytic action of thrombin has not only diagnostic and prognostic significance, but also a predicting role of NSTEMI development. The presented data provide important evidence in this context, given the association between FM and activated tenases (ICP index), circulating procoagulant phospholipases (IFP index), and increase of the inflammatory response.

Therefore, these 3 measures of hemostasis (FM, ICP and IFP) demonstrate a consistent pathophysiological association with procoagulant activation in NSTEMI patients and can be accepted as a panel of hemostasis markers with predictive value for diagnosis and prognosis. At the same time, the degree of decrease in ICP and IFP, corresponding to the FM increase, can serve as a marker for the extent of coronary arteries thrombosis, but also a predictor of the degree of cell damage, cell apoptosis, inflammation and oxidative stress. Efficient strategies for correcting these prothrombotic conditions can also be designated under this umbrella. These postulates are supported by literature data reported by other authors which demonstrated that procoagulant activity in patients with NSTEMI is closely related to the circulating level of micro-particles expressing phospholipids (derived from endotheliocytes and platelets), a fact that suggests the diagnostic feasibility of IFP estimated in this study [15, 16]. In this context, it is important to approach phosphatidylserine blockade as a tool to mitigate procoagulant activity in patients with NSTEMI, primarily in the acute phase of myocardial infarction or to prevent the development of NSTEMI in those at imminent risk.

It is also important to mention that in patients with acute coronary syndrome the level of microparticles expressing procoagulant phospholipids does not increase on the background of a moderate inflammatory response, according to the serum value of the proinflammatory markers [17]. So, it is plausible to admit that the transition of unstable angina to NSTEMI is facilitated by the activation of the procoagulant status against the background of increased inflammation and the elevation of circulating procoagulant phospholipids. The complement system activated by C-reactive protein through the alternative pathway may be involved in procoagulant activation, given the ability of the C5a component to activate CD88 receptors expressed on platelets, leukocytes and endotheliocytes, resulting in the expression of phosphotadylserine. The determination of C5a is presumptively imposed by the feasibility of predicting prothrombotic activation, and the modulation of com-

plement activity may be a tool to prevent the prothrombotic process in patients with NSTEMI.

In this study, the circulating level of hsCRP is increased, which is in agreement with the recent data report by K. Pluta et al. (2022) according to whom, on the ground of inflammation, the activation of the procoagulant system is imposed by the formation of aggregates from leukocytes and platelets (aggregation factors being the GPIIb/IIIa receptors, PSLG-1 and SD-40 ligands, as well as fibrinogen), constituting a trigger for ACS, including NSTEMI [18]. Thus, the attenuation of inflammation can also contribute to the attenuation of procoagulant activity.

Considering the trigger role of thrombin in thrombus formation, an objective of this study was to estimate the components of the anticoagulant system, which basically have the natural mission of preventing or attenuating thrombin, such as: antithrombin III, proteins C and S, and thrombomodulin. Although their role in triggering and exacerbating prothrombotic events is well argued, there are currently very few reports on the nature of their changes in patients with NSTEMI.

The obtained results indicate a significant reduction in the admission value of proteins C and S between 30 and 46%, as well as AT III by up to 20%. There are opinions, according to which the reduction of protein C below the level of 60-70% of the normal value indicates a hereditary cause of deficiency of this important anticoagulant factor [19]. The protein C regression up to 46% established in this study could plausibly be determined by 2 causes: (1) exaggerated consumption of the factor against the background of excess demand from the activated procoagulant status and/or (2) its poor synthesis in the liver.

This functional incompetence of the anticoagulant system may clearly be a mechanism for compromising the regulation of the thrombin cascade. Moreover, the impairment of these 3 anticoagulant factors at 24 and 72 hours after revascularization was directly associated with the modification of ICP, IFP and FM indices. The anticoagulant activity of factor C and cofactor S, vitamin K-dependent glycoproteins synthesized in the liver, is determined by the selective inhibition of procoagulant factors Va and VIIIa, involved in the activation of factor X and the respective formation of thrombin [20, 21]. Coronary endothelial injury accompanied by the inflammatory process decreases the expression of endothelial receptors of the annexin-V family involved in the activation of anticoagulant factor C.

Antithrombin III, synthesized in the liver, is part of the family of serum protease inhibitors, capable of inhibiting thrombin and factor Xa (the effect on factors IXa and XIa is weaker). The inhibitory activity of AT III and, respectively, the anticoagulant activity, increases, on average, by 1000 fold when it binds with heparin.

Therefore, proteins C and S, as well as AT III can be included in the panel of hemostasis markers together with ICP, IFP and FM that have predictive value for prothrombotic activity in patients with NSTEMI, in the context of detecting the pathogenetic mechanisms of the hemostasis disorder, as well as the justification of pathogenetic treatment.

The estimation of D-dimers in patients with NSTEMI did not reveal a notable change in the marker of fibrinolytic activity. Its circulating level was within the limits of the normal references of the explored method STA-Liatest and although it increased during the first 72 hours from the moment of angioplasty, it did not exceed the average value of 5 mg/ml. The presence and circulating level of D-dimers in the blood indicates the phenomenon of enzymatic degradation of the fibrin thrombus under the action of plasmin, the blood content being correlative in particular with the severity of venous thromboembolic events.

Remarkably, that J. Kim et al. (2018) found a mean concentration of D-dimers in patients with NSTEMI at the value of 5 mg/L [22], a fact that corresponds conclusively with the received data. So, it is plausible to admit that D-dimers remain, however, a specific marker of deep vein thrombosis and a predictor of the risk of pulmonary thromboembolism. However, some reports suggest that D-dimer levels are lower in NSTEMI patients than expected in STEMI patients, and these findings are understandable taking into account the difference in thrombus volume [23].

At the same time, F. Biccire et al. (2021) support the role of D-dimers as a predictor of residual thrombotic risk as well as of major cardiovascular complications in ACS patients undergoing angioplasty [24]. The blood content of D-dimers depends not only on the expression and activity of plasmin, but also on the level of some fibrinolysis inhibitors of such as alpha2-antiplasmin, lipoprotein-a, complement C3a, thrombin-triggered inhibitor of fibrinolysis, etc., while the real predictive value of D-dimers regarding hemostasis endangerment in NSTEMI, remains to be proven more consistently [25].

Attempts to explain the reduced fibrinolysis in patients with NSTEMI manifested by the absence of a notable increase in D-dimers convincingly appeal to the inhibitory role of thrombin [12, 26], which according to the changes in the explored indices (i.e., ICP and FM) is conclusively high. Thrombin induces the activation of procarboxypeptidase in the liver, which cleaves the residues carboxy-terminals of the lysine in the composition of fibrin, necessary for the functional exercise of the tissue plasminogen activator to convert the precursor into plasmin, the enzyme that ensures the fibrinolysis of the fibrin thrombus and, respectively, the increase of D-dimers. Remarkable, in this context, are the results obtained by E. Shantsila et al. (2012), demonstrating increased levels of active carboxypeptidase (also defined as thrombin-derived fibrinolysis inhibitor) in NSTEMI patients, even more significantly compared to the marker attested in STEMI patients [27]. Thus, thrombin is a double-edged sword, which on the one hand triggers fibrinogen cleavage as a decisive procoagulant process, and on the other hand inhibits the formation of plasmin to the detriment of fibrinolysis, followed by the increase of D-dimers. Indices of hemostasis, such as ICP, IFP and FM, become of a greater importance, through their estimated

value of thrombin, predictive value of prothrombitic activity, the risk of developing NSTEMI, as well as markers of diagnostic value. In addition, the pathogenetic significance of monocytes in prothrombotic activation and the risk of developing NSTEMI is currently under debate, given their ability to release extrinsic tenase trigger tissue factor, thrombin promoter and, therefore, thrombin-derived fibrinolysis inhibitor, and the fibrin thrombus formed in this way is, according to some reports, much more resistant to the action of heparin and enoxaparin [28].

So, hemostasis modifications in patients with acute coronary syndrome remain a current topic of cardiology, and from the clear finding of the role of coronary thrombus in the genesis of STEMI and NSTEMI derives several research directions regarding the assessment of: (1) predictors of prothrombosis and risk of infarction in combination with various cardiovascular risk factors; (2) diagnostic and prognostic predictors; 3) risk predictors of MACE (major cardiovascular events) in the postinfarction period; 4) efficacy of hemostasis correction therapy in relation to postinfarction clinical and functional evolution of patients with NSTEMI.

Conclusions

1. Changes in hemostasis indices estimated in patients with NSTEMI excel by significantly reducing the value of ICP, IFC and increasing more than twice fold the FM, which indicates the activation of procoagulants, as well as a decrease of up to 46% in the circulating level of markers of the anticoagulant system (proteins C, S and antithrombin III) compared to normal values.

2. The plasma content of soluble fibrin monomers was imposed in this study by the most important deviation from the normal value (>100%), compared to other markers, it directly reflects the level of thrombin that triggers the last enzymatic phase of fibrinogen cleavage and can thus be prioritized as a diagnostic predictor of prothrombotic activation and NSTEMI.

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Ethics approval and consent to participate
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Conflict of interests
No competing interests were disclosed.