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Regular paper

Decrease of prothrombin level during thrombolysis in acute myocardium infarction

Daria S. Korolova[1], Alexander M. Parkhomenko[2], Volodymyr O. Chernyshenko[1] ✉, Tamara M. Chernyshenko[1], Nadiya M. Druzhyna[1], Olha V. Hornytska[1] and Tetyana M. Platonova[1]

[1]Palladin Institute of Biochemistry, National Academy of Science of Ukraine; [2]State Institutional Scientific Center The M.D. Strazhesko Institute of Cardiology, Clinical and Regenerative Medicine of The National Academy of Medical Sciences of Ukraine

Previously, the direct interactions of Bβ26-42 fibrin residues with prothrombin were demonstrated. It was also shown that forming prothrombin complexes with E- or DDE-fragments causes non-enzymatic prothrombin activation. The direct measuring of the prothrombin level in the blood plasma of patients with acute myocardial infarction (AMI) allowed us to find a situation where such an activation can occur *in vivo*. Blood coagulation parameters in the blood plasma of patients with AMI were measured at 2 hours, three days, and seven days after the thrombolysis by streptokinase accompanied with intravenous administration of anticoagulants: unfractionated high molecular weight heparin (HMWH) and low-molecular-weight heparin (LMWH). The prothrombin level in the blood plasma of patients with AMI was normal before thrombolytic therapy and substantially decreased after streptokinase administration. This effect was prominent in the case of concomitant anticoagulant therapy with LMWH and was not observed when HMWH was applied. It can be explained by the fact that LMWH preferentially inhibits factor Xa, while the HMWH is an effective inhibitor of both factor Xa and thrombin. This observation suggested that the prothrombin level decrease was caused by the thrombin-like activity and possible autolysis of prothrombin by thrombin. Also, thrombolytic therapy with streptokinase caused the accumulation of fibrin degradation products (FDPs), some of which were able to bind prothrombin. The dramatic decrease of prothrombin level in the blood plasma of patients with AMI during thrombolysis allowed us to conclude the non-enzymatic prothrombin activation with the following autolysis of prothrombin that contributes to the pathology.

Keywords: Prothrombin, non-enzymatic activation, acute myocardial infarction, thrombosis, soluble fibrin

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✉e-mail: bio.cherv@biochem.kiev.ua
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Abbreviations: AMI, acute myocardial infarction; APTT, activated partial thromboplastin time; ATIII, antithrombin III; FX, factor X; FDPs, fibrin degradation products; HMWH, high molecular weight heparin; LMWH, low-molecular-weight heparin; PC, protein C; pNa, *p*-nitroaniline; PT, prothrombin time; RVV, Russell's viper venom; SFMCs, soluble fibrin monomeric complexes; SK, streptokinase

INTRODUCTION

Thrombotic complications are the main reason for the development of cardiovascular diseases, particularly acute myocardial infarction (AMI), which causes acute or persistent ischemic anoxia of the coronary artery. Such anoxia and the occlusion of the coronary artery are caused by intravascular thrombus formation that terminates the proper blood flow in coronary vessels (Grover *et al.*,., 2018; Karbach *et al.*,., 2019; Hudeca *et al.*,., 2020; Wieczór *et al.*,., 2020).

Activation of the blood coagulation system leads to the appearance in the bloodstream of a considerable amount of thrombin due to the activation of prothrombin. Thrombin leads to the activation of platelets and conversion of fibrinogen to fibrin monomer, which, upon polymerization, forms a fibrin clot – the core of a thrombus (Pasupathy *et al.,* 2018; Toshiaki *et al.,* 2016; Wood *et al.,* 2011).

Thrombin and prothrombin (its inactive precursor) are crucial factors in physiological and pathophysiological coagulation. A general assessment of thrombin activity and prothrombin content is necessary for proper patient management (Korolova *et al.,* 2021). However, determining the prothrombin time, which characterizes the state of the coagulation factors of the external blood clotting pathway, does not allow for determining the contribution of prothrombin in the test result. Increases in thrombin generation are being detected by the determination of prothrombin fragment 1+2, thrombin-antithrombin complex, prethrombin-1, or soluble fibrin as products of thrombin-catalyzed reactions. The total level of prothrombin in the blood plasma of patients needs to be measured (Negrier *et al.,* 2019; Wexels *et al.,* 2016; Zhao *et al.,* 2023; Elged *et al.,* 2016).

The purpose of our work was to apply the direct measuring of the level of prothrombin in the blood plasma of patients with acute myocardial infarction (AMI). To achieve this goal, we studied blood coagulation parameters in the blood plasma of patients with AMI at 2 hours, three days and seven days after the thrombolysis by streptokinase accompanied with intravenous administration of anticoagulants: unfractionated heparin and low-molecular-weight heparin (LMWH).

MATERIALS AND METHODS

Materials

Chromogenic substrates S2238 (H-D-Phe-Pip-Arg-pNA), S2765 (Z-D-Arg-Gly-Arg-pNA), and S2236 (p-Glu-Pro-Arg-pNa) were purchased from BIOPHEN (Neuville-sur-Oise, France). Factor X activator from *Russel vipera* venom (RVV), thrombin from human blood plasma, sodium citrate, phosphate-buffered saline (PBS) tablets (pH 7.2, sodium chloride, 0.15 M) were purchased from Sigma-Aldrich (St. Louis, USA). APTT-reagent, control donors' blood plasma, and test systems for determining Protein C and antithrombin III levels were purchased from Siemens-Biomed (Marburg, Germany). The thrombin-like enzyme was purified from the venom of *Agkistrodon halys halys* according to the method described in (Solovjov *et al.,* 1998). The activator of prothrombin (ecamulin) was purified from *Echis multisquamatis* venom according to the method of Solovjov and others (Solovjov *et al.,* 1996). Monomeric fibrin desAB was purified according to the method described (Pozdnjakova *et al.,* 1979).

Patients

The study included patients with large-focal and transmural AIM under 70 without signs of cardiogenic shock (n=57). The patients were treated at the State Institutional Scientific Center The M.D. Strazhesko Institute of Cardiology, Clinical and Regenerative Medicine of The National Academy of Medical Sciences of Ukraine, Kyiv.

On average, patients were taken to the division of reanimation and intensive therapy in the first 6 hours after the heart attack in 3.1±0.2 h. Patients were 57.4 years old on average. Some of them had arterial hypertension (45.5%), diabetes (7%), and 64% were smokers. The average BMI was 28.4 kg/m^2.

Inclusion criteria were clinical signs of acute coronary syndrome, registration of the elevation of the ST segment of the electrocardiogram by 2 mm or more in two measurements, age from 30 to 70 years.

Exclusion criteria were cardiogenic shock, severe forms of diabetes, bronchial asthma, kidney or liver failure, documented disorders in the blood coagulation system, bleedings during the last six months, level of hemoglobin below 100 g/l, violation of cerebral blood flow, and body weight above 110 kg or below 60 kg.

Systemic thrombolytic therapy was performed with streptokinase (SK) (Kabikinase from 'Pharmacia&Upjohn', USA) at 1500000 IU and was carried out for 30–40 minutes. All patients received basic anti-platelet therapy, therapy by beta blockers, statins, ADP inhibitors, and nitrates.

Anticoagulant prophylaxis was performed with fraxiparin or heparin 4–6 hours after the start of thrombolytic therapy. Standard unfractionated heparin was administered by an intravenous infusion lasting 48 hours (activated partial thromboplastin time (APTT was controlled), in an average dose of 1000 IU per hour, then subcutaneously four times a day for 5–7 days.

Fraxiparine ('Sanofi-Synthelabo,' France) was administered by bolus administration (0.6 ml) and then three hours later under the skin at the same dose for 3 days. Administration of Fraxiparine was then continued at half the dose until the 6–7th day of myocardial infarction.

Platelet-poor blood plasma was prepared from citrated blood by centrifugation at 1200×*g* for 30 min. Sodium citrate (3.8%) added immediately after collection to the whole blood at a 1:9 ratio was used as an anticoagulant. All work was conducted under the Declaration of Helsinki. Studies were conducted per the Ethical Committee Approval N 3 form 04.04.2019 (Palladin Institute of Biochemistry of NAS of Ukraine).

Methods

Fibrinogen concentration

Fibrinogen concentration in the blood plasma was determined by the modified spectrophotometric method. Blood plasma (0.2 ml) and PBS (1.7 ml) were mixed in a glass tube. Coagulation was initiated by adding 0.1 ml of the thrombin-like enzyme from the venom of *Agkistrodon halys halys* (1 NIH/ml), which allowed to avoid fibrin cross-linking. The mixture was incubated for 30 min at 37°C. The fibrin clot was removed and re-solved in 5 ml of 1.5% acetic acid. The concentration of protein was measured using a spectrophotometer POP (Optizen, Korea) at 280 nm (ε=1.5) (Sokolovska *et al.,* 2002).

Activated partial thromboplastin time

Activated partial thromboplastin time (APTT) was performed following the procedure: 0.1 ml of studied blood plasma was mixed with an equal volume of APTT-reagent and incubated for 3 minutes at 37°C. Then, the coagulation was initiated by adding 0.1 ml of 0.025 M solution of $CaCl_2$, and clotting time was monitored. The clotting time was evaluated using a coagulometer CT2410 (Solar, Belarus).

Prothrombin time

Prothrombin time (PT) was measured as follows: clotting was initiated by mixing 0.1 ml of blood plasma with 0.1 ml of 0.025 M $CaCl_2$ and 0.1 ml of thromboplastin reagent, time of clotting was monitored. Thromboplastin acts through the tissue factor pathway of coagulation and activates only carboxylated and uncleaved forms of prothrombin. The clotting time was evaluated using a coagulometer CT2410 (Solar, Belarus).

Prothrombin level

Total prothrombin level was measured using ecamulin (the prothrombin activator from *Echis multisqumatis* snake venom) and chromogenic thrombin-specific substrate S2238 (H-D-Phe-Pip-Arg-pNA). Ecamulin activates prothrombin and all of its inactive forms to determine total prothrombin level (Korolova *et al.,* 2023).

In a well of a 96-well plate, 0.02 ml of studied blood plasma sample, 0.03 ml of S2238 solution (0.25 mM), and 0.03 ml of ecamulin solution (0.06 mg/ml) were admixed in the PBS with 0.001 M $CaCl_2$ at final volume of 0.25 ml. The generation of colorful p-nitroaniline (pNa) was monitored at 405 nm using a ThermoMultiscan multi-plate reader (ThermoFisher, USA). Results were presented as % from control values.

Soluble fibrin monomeric complexes (SFMCs)

For the SFMCs measurement in the glass tube, the 0.25 ml of studied blood plasma sample was mixed with an equal volume of 0.1 M KH_2PO_4 buffer pH 7.5. Then 0.4 ml of 1 M KH_2PO_4 buffer pH 7.5 was added to the whole volume after gentle mixing. Samples were incubated for 30 min at ambient temperature. After incubation, the accumulation of saturated SFMCs was estimated semi-quantitatively in the range of concentrations 7–140 μg/ml by following gradation: clear solution without turbidity – negative result;

Table 1. Hemostasis system parameters of the patients with AMI at the time of admission to the clinic. The median value, minimum, and maximum are given.

Parameter	Healthy donors (n=30)	Patients with AMI (n=57)
Fibrinogen level, g/l	2.5 (2.3–3.0)	3.2* (2.2–5.5)
APTT, sec	45 (41–49)	41.0* (23–71)
Prothrombin level, %	100 (85–105)	105* (64–127)
SFMCs, μg/ml	0.0 (0.0–3.0)	40.0* (17–140)
Protein C level, %	100 (90–115)	63* (40–110)
AT III level, %	100 (90–110)	100 (62–120)
Clotting factor X level, %	100 (90–100)	92 (60–120)

*$p<0.05$, compared to donors

several dots in the solution – up to 7 μg/ml; visible turbidity – up to 35 μg/ml; formation of flakes – up to 70 μg/ml; formation of fibers – up to 90 μg/ml; gel-like sedimentation – up to 140 μg/ml. For the calibration, we used blood plasma samples with monomeric fibrin desAB added at final concentrations from 7–140 μg/ml prepared according to the method (Pozdnjakova *et al.,* 1979).

Protein C level

Protein C (PC) level was determined using the activator of PC (Berichrom, Germany). The generation of activated PC was measured by chromogenic substrate assay using specific chromogenic substrate S2236 (p-Glu-Pro-Arg-pNa). The analysis was performed in 0.05 M Tris-HCl buffer pH 7.4, at 37°C. The chromogenic substrate concentration was 30 mM. The generation of para-nitroaniline was measured at 405 nm on a microtiter plate reader Multiscan EX (Thermo Fisher Scientific, Waltham, USA). Here and below for Multiscan EX: Linearity 0–2 Abs, ± 2.0% at 405 nm; accuracy ± 2.0% or ± 0.007 Abs at 405 nm. Results were presented as the ratio of PC level in the blood plasma of the studied sample to PC level in the blood plasma of the healthy donor.

Antithrombin level

Antithrombin III (ATIII) activity is determined based on its ability to neutralize thrombin in the presence of heparin. The overabundance of thrombin was inhibited by the ATIII-heparin complex proportionally to the ATIII in blood plasma.

The analysis was performed according to the recommendations of the manufacturer. The remaining activity of thrombin was measured using chromogenic thrombin-specific substrate S2238 (H-D-Phe-Pip-Arg-pNA). Colorful *p*-nitroaniline (pNa) generation was monitored at 405 nm using ThermoMultiscan (ThermoFisher, USA). The amount of ATIII was determined using the calibration curve.

Factor X level

The total factor X level was determined using RVV (Russell's viper venom reagent that specifically activates factor X) and factor Xa-specific chromogenic substrate S22765 (Z-D-Arg-Gly-Arg-pNA). In a well of 96-well plate, 0.02 ml of studied blood plasma sample, 0.03 ml of S2765 solution (0.25 mM), and 0.01 ml of RVV solution (Sigma, USA) were admixed in the PBS with 0.001 M $CaCl_2$ at a final volume of 0.25 ml. Colorful p-nitroaniline (pNa) generation was monitored at 405 nm using ThermoMultiscan (ThermoFisher, USA). Results were presented as % of control values (Budzynski *et al.,* 2001).

Statistics

Statistical data analysis was performed using the Wilcoxon-Mann-Whitney (WMW) test using an online calculator provided by Social Science Statistics (https://www.socscistatistics.com/). Statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Results are presented as the group's median, maximum, and minimum values. All assays were performed in a series of at least three replicates. Data were considered significant when $p<0.05$.

RESULTS

Blood coagulation system parameters

An analysis of the parameters of the haemostasis system was carried out in 57 patients with great magistral transmural host myocardial infarction (AMI) at admission to the clinic (Table 1).

A significant reduction in the blood plasma clotting time in the APTT test was revealed in 49% of patients, an increase in fibrinogen content in 31% of patients, and an accumulation of SFMC (17–140 μg/ml), which indicates the activation of the blood clotting system, was determined in all studied samples. Simultaneously with the activation of the components of the coagulation system, the potential of the anticoagulant link of the system (PC and ATIII) decreases, which, together with the increased content of fibrinogen, can contribute to the increase of further thrombotic complications and re-occlusions.

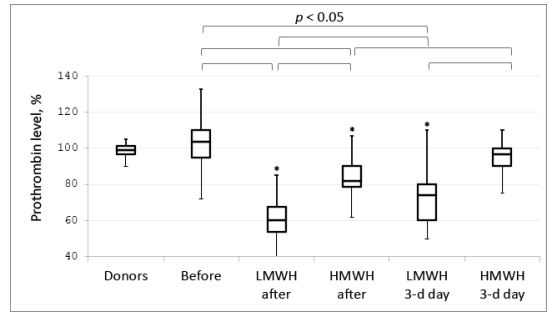

Figure 1. Level of prothrombin in the blood plasma of patients with acute myocardial infarction before thrombolysis (before) and after thrombolysis accompanied by concomitant anticoagulant therapy with low molecular weight heparin (LMWH after, n=28) or high molecular weight heparin (HMWH after, n=29). Samples were collected before, after 2 hours, and on the third day after thrombolysis. Donors – healthy donors' group (n=30). *$p\leq0.05$ in comparison to donors.

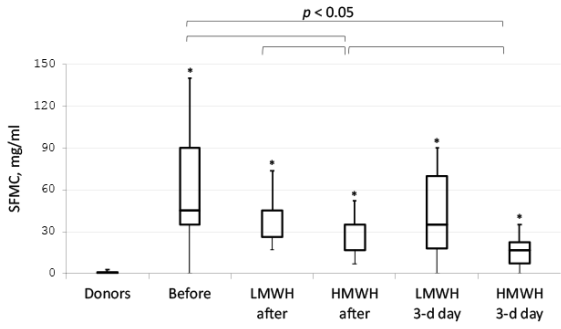

Figure 2. Level of soluble fibrin monomeric complexes (SFMC) in the blood plasma of patients with acute myocardial infarction before thrombolysis (Before) and after thrombolysis accompanied by concomitant anticoagulant therapy with LMWH (LMWH after, n=28) or HMWH (HMWH after, n=29).
Samples were collected before, after 2 hours, and on the third day after thrombolysis. Donors – healthy donors' group (n=30). *$p \leq 0.05$ in comparison to donors.

Prothrombin level after thrombolysis

The prothrombin level in the blood plasma of patients with AMI was determined before thrombolytic therapy (at the time of admission to the clinic) and after thrombolytic therapy (on the first, third, and seventh days). The prothrombin total level in the blood plasma of patients with myocardium infarction was normal (95±11%). During thrombolysis and concomitant therapy with LMWH, there was a decrease in the plasma levels of prothrombin (median 55%; min 40%, max 80%, $p<0.001$). Whereas less obvious change was observed in the case of HMWH (median 84%; min 56, max 110%, $p<0.001$) (Fig. 1). Statistic analysis in "Statistica 7" was used to calculate the correlations of total prothrombin level.

The low level of prothrombin in the case of concomitant therapy with LMWH was also found on the third day after thrombolysis. The normalization of prothrombin level was detected only on the seventh day after thrombolysis in this group of patients (median 87%; min 70, max 110%).

Correlation of prothrombin level and SFMC after thrombolysis

A decrease in the level of prothrombin in the blood plasma of patients who were injected with low molecular weight heparin (LMWH) is accompanied by a significant accumulation of SFMC (Fig. 2). In the group of patients who were injected with high molecular weight heparin (HMWH), the level of SMFC after thrombolysis was lower than in the case of LMWH injection. These data correlate to the decrease of prothrombin content at the same time points presented in Fig. 1.

DISCUSSION

A study of the blood coagulation system of patients with AIM revealed a violation of the balance between pro- and anticoagulants, which is a consequence of activating the coagulation link of the hemostasis system. The activation of the blood coagulation system is evidenced by the increased level of fibrinogen, the accumulation of SFMC, and the significant consumption of the main inhibitors – ATIII and protein C (Hudeca *et al.,* 2020; Hoshino *et al.,* 2018; Refaai *et al.,* 2018).

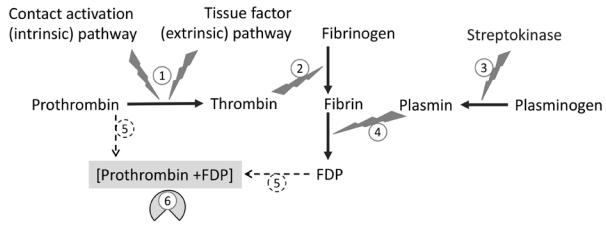

Figure 3. Scheme of prothrombin activation by fibrin degradation products during thrombolysis.
1 – canonical prothrombin activation, 2 – thrombin cleaves fibrinogen with fibrin formation, 3 – streptokinase activates plasminogen with plasmin formation, 4 – plasmin cleaves fibrin with fibrin degradation products (FDP) formation, 5 – prothrombin and FDP form complexes, 6 – prothrombin in (prothrombin-FDP) complex forms thrombin-like active site.

The accumulation of SFMC is the result of the work of the central enzyme of the blood coagulation system – thrombin (Dizhou *et al.,* 2023). The content of SFMC corresponds to the activity of thrombin and characterizes the degree of activation of the hemostasis system. There is no doubt that SFMC, as a marker of activation of the blood coagulation system, has a high prognostic value (Masahiro *et al.,* 2009).

The prothrombin level in the blood plasma of patients with AMI was normal before thrombolytic therapy and substantially decreased after streptokinase administration. This effect was prominent in the case of concomitant anticoagulant therapy with LMWH and was not observed when HMWH was applied. It can be explained by the fact that LMWH preferentially inhibits factor Xa, while the HMWH is an effective inhibitor of both factor Xa and thrombin. This observation suggested that the prothrombin level decrease was caused by the thrombin activity and possible autolysis of prothrombin by thrombin (Croles *et al.,* 2019; Makarem *et al.,* 2023; Spencer *et al.,* 2000).

Another important issue is fibrin degradation products (FDPs) accumulation due to thrombolytic therapy with streptokinase. Some of the FDPs (including DDE-complex and fibrin E-fragment) still have sites of interactions with thrombin and prothrombin (Capitanescu *et al.,* 2016) (Fig. 3).

Previously, we demonstrated the direct interactions of high molecular weight E-fragment of fibrin (E_1-fragment) and DDE-complex with prothrombin. We confirmed that prothrombin can bind to Bβ26-42 fibrin residues, and the formation of such a complex causes a non-enzymatic prothrombin activation (Chernyshenko *et al.,* 2015).

A similar complexation with the formation of a thrombin-like active site was shown for staphylocoagulase. Staphyloprothrombin, similar to prothrombin in a complex with E_1-fragment, is enzymatically active (Ashoka *et al.,* 2020; Crosby *et al.,* 2016).

As shown *in vitro*, non-enzymatic activation of prothrombin in a complex with E_1-fragment and DDE-complex was assumed as the additional mechanism of autolysis of prothrombin (Platonova *et al.,* 2002; Savchuk *et al.,* 2006). A study of the state of a hemostatic system at thrombolysis in AMI allowed us to find a pathology in which the proposed mechanism can occur *in vivo*. This suggestion was confirmed by the dramatic decrease of prothrombin level that occurs alongside the accumulation of FDPs and the inability to inhibit thrombin activity by low molecular weight heparin (LMWH).

CONCLUSIONS

The drop in total prothrombin level in the blood plasma of patients with acute myocardial infarction during thrombolysis was observed. It was strongly dependent on the type of anticoagulant therapy and was not obvious in the case of concomitant therapy with HMWH. It can be explained by the non-enzymatic activation of prothrombin by FDPs that contain Bβ26-42 residues (DDE, E_1 fragments). Such a novel type of non-enzymatic prothrombin activation was previously shown *in vitro*. Current findings allowed us to conclude the possibility of the activation *in vivo* when a huge amount of FDPs are forming. This newly discovered effect should be taken into consideration during patient management.

Declarations

Ethical Committee Approval. Palladin Institute of Biochemistry of NAS of Ukraine (#3, 04.04.2019).

Conflicts of Interest. The authors declare no conflict of interest.

REFERENCES

Budzynski AZ (2001) Chromogenic substrates in coagulation and fibrinolytic assays. *Lab Med* **32**: 365–368. https://doi.org/10.1309/KVYM-1C8E-Y6M4-MJDY

Capitanescu C, Macovei Oprescu AM, Ionita D, Dinca GV, Turculet C, Manole G, Macovei RA (2016) Molecular processes in the streptokinase thrombolytic therapy. *J Enzyme Inhibit Med Chem* **31**: 1411–1414. https://doi.org/10.3109/14756366.2016.1142985

Chernyshenko VO, Chernyshenko TM, Korolova DS, Volynets GP, Kolesnikova IN, Platonova TM, Lugovskoy EV (2015) Non-enzymatic activation of prothrombin induced by interaction with fibrin β26-42 region. *Acta Biochim Pol* **62**: 517–522. https://doi.org/10.18388/abp.2014_896

Crosby HA, Kwiecinski J, Horswill AR (2016) *Staphylococcus aureus* aggregation and coagulation mechanisms, and their function in host-pathogen interactions. *Adv Appl Microbiol* **96**: 1–41. https://doi.org/10.1016/bs.aambs.2016.07.018

Croles FN, Lukens MV, Mulder R, de Maat MPM, Mulder AB, Meijer K (2019) Monitoring of heparins in antithrombin-deficient patients. *Thrombosis Res* **175**: 8–12. https://doi.org/10.1016/j.thromres.2019.01.007

Elged AA, El-Gamal RA, Bastawy SA, Moselhy MS (2016) Soluble fibrin monomer complex assay enhances early and accurate diagnosis of acute myocardial infarction. *Int J Clin Exp Pathol* **9**: 5801–5809

Grover SP, Mackman N (2018) Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. *Arterioscl Thromb Vascul Biol* **38**: 709–725. https://doi.org/10.1161/ATVBAHA.117.309846

Hoshino K, Muranishi K, Kawano Y, Hatomoto H, Yamasaki S, Nakamura Y, Ishikura H (2018) Soluble fibrin is a useful marker for predicting extracorporeal membrane oxygenation circuit exchange because of circuit clots. *J Artificial Organs* **21**: 196–200. https://doi.org/10.1007/s10047-018-1021-x

Hudec S, Hutyra M, Precek J, Latal J, Nykl R, Spacek M, Sluka M, Sanak D, Tudos Z, Navratil K, Pavlu L, Taborsky M (2020) Acute myocardial infarction, intraventricular thrombus and risk of systemic embolism. *Biomed Papers Med Faculty of the University Palacky, Olomouc, Czechoslovakia* **164**: 34–42. https://doi.org/10.5507/bp.2020.001

Iba T, Ito T, Maruyama I, Jilma B, Brenner T, Müller MC, Juffermans NP, Thachil J (2016) Potential diagnostic markers for disseminated intravascular coagulation of sepsis. *Blood Rev* **30**: 149–155. https://doi.org/10.1016/j.blre.2015.10.002

Ieko M, Naito S, Yoshida M, Kanazawa K, Mizukami K, Sato H, Yui T, Nakabayashi T, Hirabayashi T, Oguma Y (2009) Plasma soluble fibrin monomer complex as a marker of coronary thrombotic events in patients with acute myocardial infarction. *Tohoku J Exp Med* **219**: 25–31. https://doi.org/10.1620/tjem.219.25

Karbach S, Lagrange J, Wenzel P (2019) Thromboinflammation and vascular dysfunction. *Hamostaseologie* **39**: 180–187. https://doi.org/10.1055/s-0038-1676130

Korolova D (2021) Regulation and dysregulation of thrombin activity. *Southeastern Eur Med J* **5**: 47–64. https://doi.org/10.26332/seemedj.v5i1.194

Korolova D, Gryshchenko V, Chernyshenko T, Platonov O, Hornytska O, Chernyshenko V, Klymenko P, Reshetnik Y, Platonova T (2023) Blood coagulation factors and platelet response to drug-induced hepatitis and hepatosis in rats. *Anim Models Exp Med* **6**: 66–73. https://doi.org/10.1002/ame2.12301

Maddur AA, Kroh HK, Aschenbrenner ME, Gibson BHY, Panizzi P, Sheehan JH, Meiler J, Bock PE, Verhamme IM (2020) Specificity and affinity of the N-terminal residues in staphylocoagulase in binding to prothrombin. *J Biol Chem* **295**: 5614–5625. https://doi.org/10.1074/jbc.RA120.012588

Makarem A, Zareef R, Abourjeili J, Nassar JE, Bitar F, Arabi M (2023) Low molecular weight heparin in COVID-19: benefits and concerns. *Frontiers Pharmacol* **14**: 1159363. https://doi.org/10.3389/fphar.2023.1159363

Negrier C, Shima M, Hoffman M (2019) The central role of thrombin in bleeding disorders. *Blood Rev* **38**: 100582. https://doi.org/10.1016/j.blre.2019.05.006

Pasupathy S, Rodgers S, Tavella R, McRae S, Beltrame JF (2018) Risk of thrombosis in patients presenting with myocardial infarction with nonobstructive coronary arteries (MINOCA). *TH Open: Comp J Thrombosis Haemos* **2**: e167–e172. https://doi.org/10.1055/s-0038-1645875

Platonova TM, Slominskyi OI, Chernyshenko TM, Makohonenko EM (2002) Activation of key proenzymes of the blood coagulation system by the fibrin E fragment. *Ukrain Biochem J* **74**: 25–29

Pozdnjakova TM, Musjalkovskaja AA, Ugarova TP, Protvin DD, Kotsjuruba VN (1979) On the properties of fibrin monomer prepared from fibrin clot with acetic acid. *Thrombosis Res* **16**: 283–288. https://doi.org/10.1016/0049-3848(79)90292-5

Refaai MA, Riley P, Mardovina T, Bell PD (2018) The clinical significance of fibrin monomers. *Thrombosis Haemostasis* **118**: 1856–1866. https://doi.org/10.1055/s-0038-1673684

Solovjov DA, Ugarova TP (1993) Purification and characterization of α-specific thrombin-like enzymes from the venoms of middle asian pit viper snakes *Agkistrodon halys halys* and *Agkistrodon halys blomhoffii*. *Biochemistry (Moscow)* **58**: 1221–1233

Solovjov DA, Platonova TN, Ugarova TP (1996) Purification and characterization of ecamulin – A new prothrombin activator from the *Echis multisquamatus* snake venom. *Biochemistry (Moscow)* **61**: 785–793

Sokolovska AS, Chernyshenko TM, Ivanenko TI (2002) Comparative characteristic of fibrinogen level determination methods in blood plasma. *Exper Clin Phichem* **3**: 82–86

Spencer FA, Ball SP, Zhang Q, Liu L, Benoit S, Becker RC (2000) Enoxaparin, a low molecular weight heparin, inhibits platelet-dependent prothrombinase assembly and activity by factor-Xa neutralization. *J Thrombosis Thrombolysis* **9**: 223–228. https://doi.org/10.1023/a:1018710526772

Savchuk OM, Krasnobryzha IM, Horchev VF, Chernyshenko TM, Havryliuk, SP, Platonova TM (2006) Prothrombine and E-fragment complexation process. *Ukrainian Biochem J* **78**: 99–105 (in Ukrainian)

Wexels F, Seljeflot I, Pripp AH, Dahl OE (2016) D-Dimer and prothrombin fragment 1+2 in urine and plasma in patients with clinically suspected venous thromboembolism. *Blood Coagul Fibrinol: Int J Haemostasis Thrombosis* **27**: 396–400. https://doi.org/10.1097/MBC.0000000000000461

Wieczór R, Kulwas A, Rość D (2020) Implications of hemostasis disorders in patients with critical limb ischemia-an in-depth comparison of selected factors. *J Clin Med* **9**: 659. https://doi.org/10.3390/jcm9030659

Wood JP, Silveira JR, Maille NM, Haynes LM, Tracy PB (2011) Prothrombin activation on the activated platelet surface optimizes expression of procoagulant activity. *Blood* **117**: 1710–1718. https://doi.org/10.1182/blood-2010-09-311035

Wu D, Prem A, Xiao J, Salsbury FR (2023) Thrombin - A Molecular Dynamics Perspective. *Mini Rev Med Chem*. Advance online publication. https://doi.org/10.2174/1389557523666230821102655

Zhao X, Yang S, Lei R, Duan Q, Li J, Meng J, Sun L (2023) Clinical study on the feasibility of new thrombus markers in predicting massive cerebral infarction. *Front Neurol* **13**: 942887. https://doi.org/10.3389/fneur.2022.942887