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LONG-TERM THROMBOTIC EVENTS IN PATIENTS WITH MYOCARDIAL INFARCTION: PROTEOMIC ANALYSIS OF PLATELETS AND PLASMA COMBINED WITH HEMOSTASIS ASSESSMENT

<i>Aim</i>	To determine factors associated with an increased risk of thrombotic complications during antiplatelet therapy in patients with acute myocardial infarction (AMI) based on a study of the parameters of hemostasis functional activity, as well as the proteomic composition of blood plasma and platelets.
<i>Material and methods</i>	A single-center prospective clinical study assessed hemostasis parameters and quantified the plasma and platelet proteome before and after dual antiplatelet therapy (DAPT) in 98 AMI patients. Clinical data on thrombotic complications that occurred during the follow-up period were collected for 12 months after the inclusion into the study. A statistical analysis of the collected data was performed, and the proteins were identified whose concentrations were associated with an increased risk of late thrombotic complications.
<i>Results</i>	The comparative analysis of patient groups with and without thrombotic complications revealed statistically significant differences in the concentrations of several proteins. The univariate regression analysis identified proteins associated with an increased risk of thrombotic complications in the long term. Statistically significant factors with a 95% confidence interval different from 0 were the concentrations of platelet proteins, including von Willebrand factor, complement component C8 β -chain, and cation-independent mannose-6 phosphate receptor.
<i>Conclusion</i>	The study identified proteins whose concentrations were associated with an increased risk of thrombotic complications in AMI patients taking DAPT. Before implementing these findings in clinical practice, a randomized prospective study should be performed for validation of the study results.
<i>Keywords</i>	Antiplatelet therapy; inflammation; acute myocardial infarction; proteomic analysis; platelets; von Willebrand factor
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Introduction

Antithrombotic therapy is the cornerstone of thrombotic complication prevention and treatment in patients with acute myocardial infarction (AMI). However, large-scale studies have demonstrated that antiplatelet therapy does not completely eliminate the risk of thrombotic complications [1, 2]. Diagnosis of increased risk for thrombotic complications during antiplatelet treatment remains a pressing issue in modern cardiology. Current methods for monitoring the effectiveness

of antiplatelet therapy do not have an adequate prognostic value to be implemented in common clinical practice [1–6]. Therefore, studies aimed at identifying factors associated with an increased risk of thrombotic complications during antiplatelet therapy are in demand. Promising candidates for such factors should primarily be sought among the parameters of tests for hemostasis functional activity that have not previously been considered in assessing the risk. Furthermore, changes in protein concentrations may serve as markers

of an unfavorable prognosis, as proteins are key regulators and mediators of physiological processes in living organisms, including the hemostatic system [7]. Identification of new markers for increased risk of thrombotic complications in patients with AMI may facilitate the development of new approaches to detect the failure of antiplatelet therapy.

Aim

The aim of the study was to identify factors associated with an increased risk of thrombotic complications during the antiplatelet treatment in AMI patients based on the analysis of hemostasis parameters and plasma and platelet proteome.

Material and methods

Study design

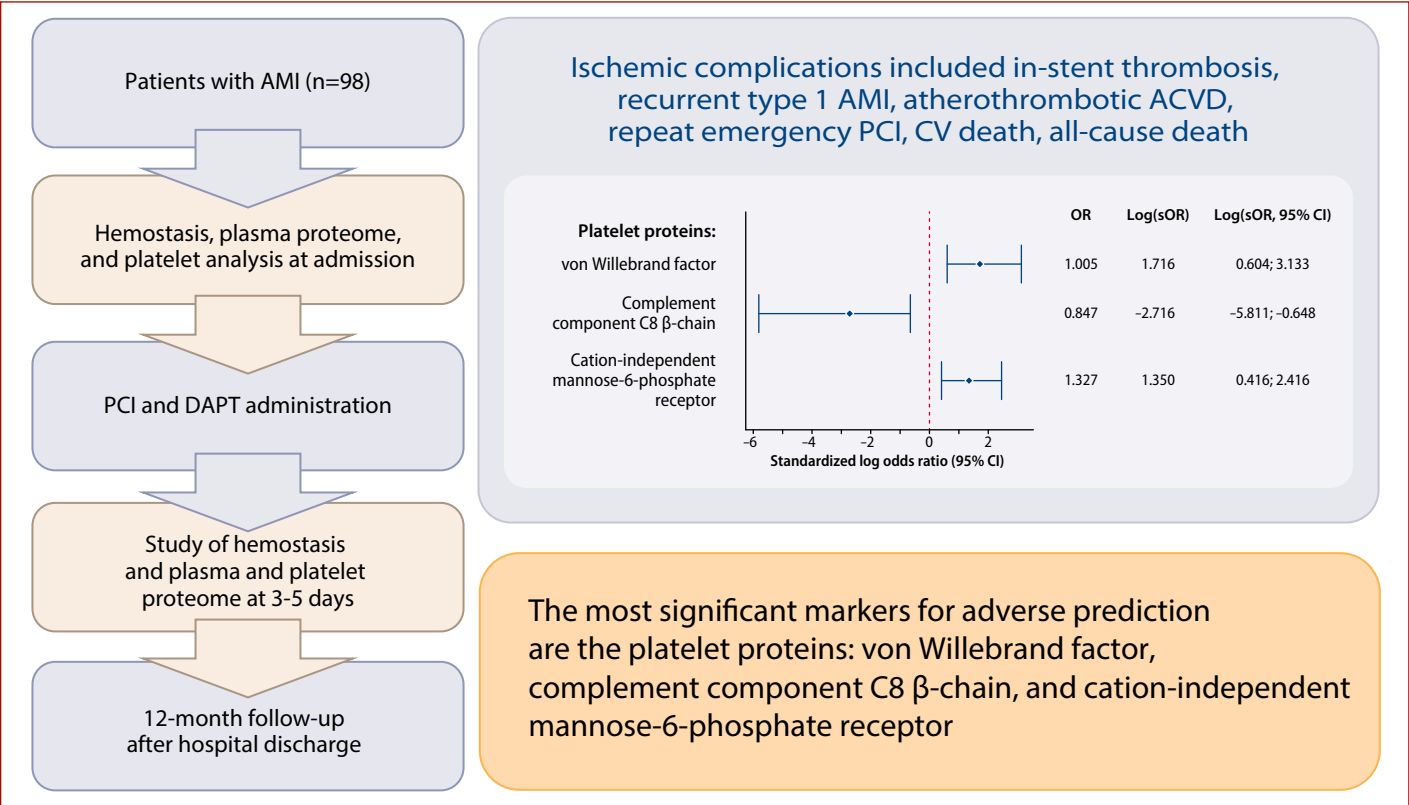
This single-center prospective study included 98 patients with ST-segment elevation (n=87) and non-ST-segment elevation (n=11) AMI admitted to the cardiac intensive care unit of the I. V. Davydovsky City Clinical Hospital of the Moscow Health Department from March 2023 through November 2024. All included patients underwent coronary angiography (CA) and per-

cutaneous coronary intervention (PCI), and were prescribed dual antiplatelet therapy (DAPT). Patients with ST-segment elevation AMI were included in the study upon admission before PCI and administering a loading dose of antiplatelet agents during the hospital stay. Patients with non-ST-segment elevation AMI were included in the study after confirming the diagnosis of AMI before PCI and administering a loading dose of antiplatelet agents, also during the hospital stay. All patients received drug therapy in accordance with current Russian and European guidelines for the treatment of ST-segment elevation MI and non-ST-segment elevation MI [8–10]. The study was performed in consistency with the principles of the Declaration of Helsinki and approved by the local ethics committee (Protocol #124 dated May 16, 2023).

Inclusion criteria:

- Diagnosis of AMI consistent with the criteria of the Fourth Universal Definition of myocardial infarction (2018) [11];
- providing a written informed consent for participation in the study.
- Non-inclusion criteria:
- Oncological disease at the inclusion in the study;
- Autoimmune disease;

Central illustration. Long-term thrombotic complications in patients with acute myocardial infarction: Results of proteomic analysis of platelets, blood plasma and hemostasis parameters



AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; DAPT, dual antiplatelet therapy; ACVD, acute cerebrovascular disease; OR, odds ratio; sOR, standardized odds ratio; CI, confidence interval

- Active inflammatory process in any location at the inclusion in the study;
- Requirement for anticoagulant therapy or receiving anticoagulant therapy at the inclusion in the study;
- Shock/clinical death on admission.

Exclusion criteria:

- Failure to comply with the study protocol;
- absence of coronary atherosclerosis confirmed by CAG data;
- choice of coronary artery bypass grafting as the optimal revascularization strategy;
- refusal of PCI in favor of conservative treatment;
- emergence of indications for anticoagulant therapy during the inpatient treatment;
- complicated course of AMI.

A standard clinical examination was performed for all patients upon inclusion in the study: complete blood count and biochemistry, coagulogram, and echocardiography (EchoCG). The functional activity of various components of the hemostasis system was also studied (rotational thromboelastometry, the Thrombodynamics test with assessment of endogenous lysis activity, impedance aggregometry). A chromatographic-mass spectrometric analysis of blood plasma, and for 61 patients, platelets (stage 1), was performed. On days 3–5 following PCI and the administration of DAPT, the study of hemostasis parameters and a chromatographic-mass spectrometric analysis of blood plasma and platelets (stage 2) was repeated for all patients.

After the discharge from the hospital, patients were followed up for 12 months with collection of data on ischemic complications. Ischemic complications included in-stent thrombosis, recurrent type 1 MI, acute cerebrovascular disease (ACVD), repeat emergency PCI, cardiovascular death, and all-cause death.

Method of blood collection

Blood samples were collected by standard venipuncture using a 21G Vacuette needle. Blood was collected in S-Monovette (Sarstedt, Germany) vacuum tubes containing ethylenediaminetetraacetic acid (EDTA), 3.2% sodium citrate (1:10), and a clotting activator.

Whole venous blood samples collected in 3.2% sodium citrate tubes were used for hemostasis functional activity tests and to obtain platelet concentrate. Plasma samples for mass spectrometry were obtained from venous blood collected in EDTA tubes.

Rotational thromboelastometry

The study was conducted on a ROTEM device (The Tem Innovations, GmbH, Germany) in NATEM mode by the standard method [12]. The following pa-

rameters were analyzed: CT (clotting time, s), CFT (clot formation time, s), α (α -angle, °), MCF (maximum clot firmness, mm), A (clot amplitude or density, mm), ML (maximum lysis, %), LI (lysis index, %).

Impedance aggregometry

The study was performed on a Multiplate apparatus (Roche) according to the standard method [13]. Arachidonic acid, adenosine diphosphate (ADP), and thrombin receptor activator peptide-6 (TRAP-6) were used as specific platelet receptor activators. Platelet aggregation activity was expressed in standard units (U).

Thrombodynamics test with assessment of endogenous lysis activity

The test was performed on platelet-free plasma samples using a Thrombodynamics Analyzer (Hemacore) according to the standard method [14]. Platelet-free plasma was obtained using a previously described method [14]. The clot growth parameters were recorded for 30 minutes during the Thrombodynamics test. When assessing the endogenous lysis activity, the clot formed around the insert was subjected to further lysis; the test time was 4 h. The following parameters were analyzed: V (clot growth rate, $\mu\text{m}/\text{min}$), V_i (initial clot growth rate, $\mu\text{m}/\text{min}$), Tlag (time lag, clot growth delay, min), CS (clot size, fibrin clot size, μm), D (density, rel. units), Tsp (time spontaneous, time of spontaneous clot emergence onset, min), LOT (lysis onset time, min), LP (lysis progression, clot lysis intensity, $\%/ \text{min}$), LTE (lysis time estimation, expected clot lysis time, min), CLT (clot lysis time, clot, min).

Method for obtaining blood plasma and platelet samples for proteomic analysis

To obtain plasma samples, whole venous blood was centrifuged at 1000 g for 10 min at room temperature. Platelet concentrate was obtained as described previously [15]. The volume of whole venous blood used to obtain platelet concentrate from one patient was 5 ml. Whole blood samples were centrifuged at 800 g for 15 min at room temperature. The plasma samples obtained by centrifugation were centrifuged again at 1800 g for 15 min at room temperature. The pellet obtained from the second centrifugation was dissolved in 1 ml of phosphate buffered saline (PBS), after which it was centrifuged for the third time at 800 g for 15 min at room temperature. The liquid portion of the plasma was then removed. The obtained plasma and platelet concentrate samples were stored at -60°C until the mass spectrometric analysis.

Proteomic analysis

Proteomic analysis of plasma and platelet fraction was performed by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS) in multiple reaction monitoring (MRM) mode. A set of synthetic stable isotope-labeled peptide standards (SIS) was used for quantitative analysis. These standards were added to each hydrolysate sample as an internal standard for measuring the respective plasma proteins. For analysis, 10 µl of each sample were injected into a reversed-phase Zorbax Eclipse Plus (RP-UHPLC) column (2.1 × 150 mm, particle diameter 1.8 µm; Agilent) using an ExionLC HPLC system (ThermoFisher Scientific, USA). Peptides were separated at a flow rate of 0.4 ml/min for 60 min by a multistep gradient. The parameters for HPLC–MS analysis were adapted and optimized based on previous studies [16]. A total of 103 plasma proteins and 129 platelet fraction proteins were analyzed by mass spectrometry.

Statistical analysis

Statistical analysis and visualization of the study data were performed in the R programming environment (version 4.3.2). Differences and associations were considered statistically significant at $p < 0.05$.

The distribution of quantitative variables was tested for normality using the Shapiro-Wilk test. Since the distribution of all quantitative values was significantly different from normal, the data were presented as the median (Me) and interquartile range [Q1; Q3], where Q1 is the 1st quartile and Q3 is the 3rd quartile. To compare two independent groups (e.g., between groups with and without ischemic complications), the Mann-Whitney U test was used. Two dependent samples (values within one group at different time points) were compared using the Wilcoxon W test for related samples.

Qualitative (categorical) variables presented in 2x2 contingency tables were analyzed using a two-tailed Fisher's exact test. To assess the effect size, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

To identify predictors of ischemic complications and assess their contribution, a series of univariate binomial logistic regression models was constructed, in which the binary outcome (presence/absence of an event) served as the dependent variable. The results of the regression analysis are presented in two forms. First, to compare the effect strength for various predictors, continuous variables were standardized using a robust z-score with the formula: $(x - \text{Me})/\text{IQR}$ (interquartile range). The results of this analysis are presented as regression coefficients with 95% CI. Second, to assess clinical signif-

Table 1. Clinical and demographic characteristics of patients included in the study

Parameter	Group 1 (n=89)	Group 2 (n=9)	P
History			
Age, years	60.5 [53; 67.75]	55 [51; 69]	0.72
Male gender	57 (64.0)	7 (77.8)	0.72
Arterial hypertension	74 (83.1)	7 (77.8)	0.39
Type 2 diabetes mellitus	4 (4.5)	3 (33.3)	0.19
Smoking	39 (43.8)	4 (44.4)	1
BMI, kg/m ²	28.81 [26.08; 35.64]	29.14 [27.14; 30.19]	0.77
History of AIM	7 (7.8)	2 (22.2)	0.20
History of PCI	6 (6.7)	1 (11.1)	0.51
History of ACVD	7 (8.2)	0	1
Outpatient therapy			
Acetylsalicylic acid	13 (14.6)	1 (11.1)	1
Statins	9 (10.1)	1 (11.1)	1
Beta blockers	10 (11.2)	1 (11.1)	1
Treatment at the EMC stage			
Acetylsalicylic acid	65 (73.0)	7 (77.8)	0.65
Clopidogrel	27 (30.3)	2 (22.2)	0.72
Morphin	25 (28.1)	1 (11.1)	0.27
P2Y12 receptor blocker after PCI			
Clopidogrel	6 (6.7)	1 (11.1)	0.53
Ticagrelor	59 (66.3)	7 (77.8)	1
Prasugrel	3 (3.4)	0	1
Clinical and procedural features			
Pain-to-balloon time, min	265 [170; 990]	270 [106; 777]	0.52
Door-to-balloon time, min	35 [31; 49]	42 [36; 49]	0.33
ST-segment elevation AMI	73 (82.0)	7 (77.8)	0.30
Echocardiography			
LVEF	50 [44; 57.75]	47 [46; 54]	0.42
Laboratory tests			
Hemoglobin, g/l	146 [128; 150]	140 [128; 150]	0.22
Leukocytes, 10 × ⁹ /l	9.34 [7.51; 11.5]	12.16 [7.84; 12.6]	0.38
Platelets, 10 × ⁹ /l	240 [208.25; 273]	222 [185; 245]	0.32
Creatinine, µmol/l	82 [73; 101]	70 [55; 89]	0.04

Values of quantitative variables are presented as median and interquartile range (Me [Q1; Q3]), and, for categorical variables, as the number of patients and their proportion in per cent (n (%)). BMI, body mass index; AMI, acute myocardial infarction; ACVD, acute cerebrovascular disease; EMC, emergency medical care; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention. Values in bold indicate $p < 0.05$.

Table 2. Comparison of tests results for hemostasis functional activity

Параметр	Values				p			
	Stage 1		Stage 2		Group 1 vs. Group 2		Stage 1 vs. Stage 2	
	Group 1	Group 2	Group 1	Group 2	Stage 1	Stage 2	Group 1	Group 2
Impedance aggregometry								
AUC Asa, U	24.00 [14.00; 36.00]	20.00 [13.00; 26.00]	8.00 [3.00; 13.00]	9.00 [2.00; 11.00]	0.360	0.670	<0.001	0.050
AUC ADP, U	33.00 [25.00; 45.00]	27.00 [26.00; 33.00]	12.00 [7.00; 16.00]	12.00 [9.00; 31.00]	0.258	0.379	<0.001	0.018
AUC TRAP-6, U	57.00 [45.00; 78.00]	47.00 [44.00; 52.00]	49.00 [37.00; 63.00]	40.00 [36.00; 50.00]	0.090	0.553	<0.001	0.726
Rotational thromboelastometry								
A10, mm	44.50 [37.00; 51.00]	42.00 [41.00; 44.00]	48.00 [41.50; 55.00]	51.00 [46.00; 53.00]	0.655	0.540	<0.001	0.058
A15, mm	51.50 [45.00; 57.00]	48.00 [47.00; 50.00]	55.00 [49.50; 60.50]	57.00 [54.00; 61.00]	0.481	0.456	<0.001	0.038
A20, mm	55.00 [49.00; 59.00]	51.00 [50.00; 53.00]	58.00 [53.00; 63.00]	59.00 [58.00; 63.00]	0.417	0.473	<0.001	0.028
A25, mm	55.50 [51.00; 60.00]	54.00 [52.00; 55.00]	59.00 [54.50; 63.50]	60.00 [59.00; 64.00]	0.506	0.456	<0.001	0.033
A30, mm	56.00 [52.00; 61.00]	55.00 [52.00; 56.00]	59.00 [55.00; 63.50]	62.00 [59.00; 63.00]	0.553	0.416	<0.001	0.027
MCF, mm	56.00 [52.00; 61.00]	55.00 [53.00; 56.00]	60.00 [55.00; 64.00]	62.00 [59.00; 64.00]	0.510	0.494	<0.001	0.044
Li60, %	93.00 [90.00; 96.00]	94.00 [93.00; 95.00]	91.00 [88.00; 93.00]	91.00 [90.00; 92.00]	0.627	0.896	<0.001	0.106
ML, %	24.00 [21.00; 28.00]	24.00 [23.00; 25.00]	26.00 [23.00; 29.50]	25.00 [24.00; 28.00]	0.831	1.000	<0.001	0.282
CLT, min	24.50 [18.05; 32.55]	20.20 [16.42; 22.75]	24.15 [16.38; 30.22]	29.40 [26.95; 32.42]	0.142	0.095	0.250	0.016
Thrombodynamics test with assessment of endogenous lysis								
V, $\mu\text{m}/\text{min}$	36.75 [31.73; 43.55]	32.20 [29.00; 34.80]	31.40 [29.20; 35.10]	31.60 [30.10; 32.00]	0.149	0.420	<0.001	0.363
Vi, $\mu\text{m}/\text{min}$	61.05 [56.60; 64.65]	57.90 [55.80; 62.00]	58.40 [55.10; 62.15]	59.50 [55.00; 62.30]	0.311	0.820	<0.001	0.820
Vst, $\mu\text{m}/\text{min}$	35.20 [30.92; 40.10]	32.15 [28.75; 34.80]	31.40 [29.20; 34.70]	31.60 [30.10; 32.00]	0.141	0.485	0.001	0.673
CS, μm	1330.50 [1198.75; 1423.00]	1251.50 [1174.50; 1299.25]	1246.00 [1177.50; 1317.00]	1263.00 [1224.00; 1292.00]	0.306	0.970	0.002	0.844
D, conv. units	27434.00 [23862.00; 30854.00]	26736.00 [24053.00; 28022.00]	29518.00 [27097.50; 32733.50]	30492.00 [27986.00; 33073.00]	0.658	0.600	<0.001	0.164
Tsp, min	29.00 [18.80; 48.50]	33.55 [28.05; 51.12]	30.40 [21.30; 44.70]	60.80 [50.27; 71.62]	0.239	0.091	0.023	0.346
Li, %	52.00 [31.70; 56.80]	46.65 [40.50; 50.22]	46.85 [31.28; 54.42]	50.25 [35.90; 54.57]	0.730	0.563	0.024	0.688
LP, Vst, $\mu\text{M}/\text{min}$	3.60 [2.70; 7.50]	4.70 [3.95; 5.95]	4.60 [3.18; 8.72]	3.95 [3.08; 6.88]	0.692	0.474	0.039	0.688

The table presents parameters with significant intergroup differences within the stages and over time. The data are presented as median and interquartile range Me [Q1; Q3]. A, amplitude; CLT, clot lysis time; CS, clot size; D, density; LI, lysis index; LP, lysis progression; MCF, maximum clot firmness; ML, maximum lysis; Tsp, time spontaneous; V, clot growth rate; Vi, initial clot growth rate. Values in bold indicate $p < 0.05$.

icance and interpretation in natural units, models were constructed on unstandardized data, the results of which are presented as OR.

Results

Main characteristics of patients

Based on the observation of ischemic complications outside the hospital, patients were divided into two groups:

Group 1, patients without ischemic complications outside the hospital ($n=89$);

Group 2, patients with ischemic complications in the hospital ($n=9$).

There were no statistically significant differences between the groups in key clinical and demographic characteristics, with the exception of creatinine concentrations, which, however, were within the reference range in both groups (Table 1).

Study of hemostasis parameters

We did not find statistically significant differences in hemostatic parameters between the study groups at any stage of the study (see Table 2; see Supplementary Materials on the journal website: Table S1). However, it should be noted that in both Group 1 and Group 2 at the second stage of the study (on days 3–5 after the ad-

Table 3. Results of comparative analysis of blood plasma proteome: the table shows proteins differentially represented between groups

Parameter	Values, fmol/μL				p			
	Stage 1		Stage 2		Group 1 vs. Group 2		Stage 1 vs. Stage 2	
	Group 1	Group 2	Group 1	Group 2	Stage 1	Stage 2	Group 1	Group 2
α ₁ -acid glycoprotein	7 979.00 [6 516.20; 9 900.10]	6 277.70 [5 186.25; 7 812.75]	10 510.00 [9 157.88; 12 460.00]	9 447.55 [8 287.70; 13 090.88]	0.032	0.497	<0.001	0.031
Apolipoprotein B100	200.60 [138.87; 277.65]	178.96 [124.34; 370.84]	219.41 [133.90; 303.15]	327.77 [259.12; 397.62]	1.000	0.038	0.924	0.093
Attractin	112.26 [98.39; 123.53]	95.64 [92.28; 97.72]	104.00 [93.07; 114.38]	99.04 [81.40; 109.30]	0.027	0.305	<0.001	0.843
β ₂ microglobulin	106.10 [86.75; 126.23]	77.57 [69.60; 90.76]	109.70 [97.07; 139.42]	103.21 [91.39; 121.53]	0.009	0.351	<0.001	0.063
Carboxypeptidase N catalytic subunit	103.12 [94.92; 122.78]	86.76 [74.00; 102.06]	124.61 [108.45; 146.10]	109.89 [99.02; 112.52]	0.017	0.056	<0.001	0.031
Ceruloplasmin	2 987.20 [2 697.00; 3 601.90]	2 638.30 [2 432.93; 2 770.27]	3 346.00 [3 004.40; 3 829.70]	3 028.05 [2 692.05; 3 367.57]	0.021	0.251	<0.001	0.063
Hemoglobin subunit α	520.78 [410.62; 699.81]	751.72 [278.92; 1765.52]	538.64 [416.56; 986.80]	1056.89 [636.4; 1467.35]	1.000	0.046	0.377	0.686
Immunoglobulin μ-chain C-domain	4 997.40 [3 573.00; 7 351.70]	4 870.20 [3 984.22; 5 952.60]	5 538.55 [4 062.50; 7 694.25]	3 563.15 [2 696.33; 4 314.33]	0.731	0.046	<0.001	0.031
Neuropilin 2	54.10 [44.13; 61.89]	45.81 [37.87; 51.60]	59.52 [48.92; 66.94]	54.99 [50.67; 60.27]	0.025	0.351	<0.001	0.094
Phospholipid transfer protein	34.05 [29.65; 40.29]	35.76 [34.96; 41.63]	27.76 [22.66; 32.61]	33.36 [32.84; 35.12]	0.226	0.042	<0.001	0.156

Data are presented as median and interquartile range Me [Q1; Q3]. Values in bold indicate p<0.05.

ministration of DAPT), the aggregometry values during stimulation with arachidonic acid and ADP corresponded to the target values during the antiplatelet therapy [17].

When examining the parameters of functional hemostasis activity over time between study stages 1 and 2, aggregometry values with ADP stimulation decreased in both groups. Furthermore, by study stage 2, increases in clot density were noted in both groups, as measured by rotational thromboelastometry. Group 1 showed an increase in the endogenous lysis activity and a decrease in the clot growth rate at stage 2 of the study according to the Thrombodynamics test, which was not observed in Group 2 (see Table 2; Supplementary Materials on the journal website: Table S1).

Evaluation of the proteome of blood plasma and platelet fraction

Further we compared the abundance of proteins in plasma and platelet fractions between the groups at each stage. We found that groups 1 and 2 differed significantly in the concentrations of 10 plasma proteins (Table 3) and 27 platelet fraction proteins (Table 4). Most of these proteins are representatives of the following functional groups: proinflammatory proteins, including acute phase proteins (α₁ acid glycoprotein, attractin, β₂ microglobulin, carboxypeptidase N catalytic subunit, immunoglobulin μ-chain C-domain, CD40 ligand, P-selectin, protein S100 A9); complement components (β-chain of the complement component C8, C8β); hemostasis system proteins (platelet endotheli-

Table 4 (start). Comparative analysis of platelet fraction proteins: the table shows proteins differentially represented between groups

Parameter	Values, fmol/ μ L				p			
	Stage 1		Stage 2		Group 1 vs. Group 2		Stage 1 vs. Stage 2	
	rpynna 1	rpynna 2	rpynna 1	rpynna 2	Stage 1	Stage 2	Group 1	Group 2
Aortic smooth muscle actin	139 840.00 [99 467.25; 190 105.00]	180 520.00 [172 875.00; 280 895.00]	128 235.00 [76 854.25; 172 427.50]	273 090.00 [214 960.00; 305 435.00]	0.008	0.008	0.424	0.219
β_2 microglobulin	1137.30 [796.57; 1677.18]	1809.70 [1435.35; 2416.90]	1263.25 [771.16; 1716.45]	2 724.90 [1 752.35; 3 003.45]	0.026	0.016	<0.001	0.109
Cation-independent mannose-6-phosphate receptor (CI-M6PR)	4.11 [2.93; 6.41]	9.39 [7.99; 10.23]	3.96 [2.92; 6.28]	9.43 [7.72; 12.55]	0.009	0.005	0.987	0.813
CD40 ligand	40.16 [23.16; 52.91]	46.87 [36.56; 87.76]	44.34 [30.50; 50.76]	85.09 [47.90; 97.19]	0.177	0.045	0.147	0.078
Coagulation factor V	357.64 [193.30; 586.21]	613.28 [397.64; 778.41]	353.67 [210.97; 510.93]	705.99 [526.32; 929.26]	0.042	0.018	0.199	0.469
Complement component C8 β -chain	20.19 [12.92; 39.76]	23.81 [17.39; 29.18]	20.64 [13.34; 30.45]	11.80 [6.94; 15.29]	0.932	0.011	0.239	0.047
Cystatin C	33.64 [25.36; 43.89]	62.36 [45.31; 70.60]	43.44 [28.21; 54.54]	68.42 [51.11; 96.49]	0.016	0.030	<0.001	0.219
Extracellular matrix protein 1	36.77 [29.83; 45.15]	44.66 [40.46; 53.09]	38.31 [28.76; 45.96]	52.29 [44.56; 64.08]	0.117	0.038	0.296	0.297
Fibrinogen α -chain	16 860.00 [13 913.00; 21 481.00]	16 078.00 [13 534.75; 17 882.00]	12 672 [10 149.50; 15 264.00]	19 016.00 [15 634.00; 20 369.00]	0.345	0.042	0.007	0.156
Fibrinogen β -chain	24131.00 [18 960.00; 29 178.00]	20 705.50 [17 518.50; 27 932.25]	17 319.50 [14 474.25; 22 466.25]	29 210.00 [22 789.50; 31 304.00]	0.412	0.017	0.028	0.078
Fibrinogen γ -chain	12 489.00 [10 484.00; 15 741.00]	12 481.00 [9 955.45; 13 416.50]	9 628.40 [8 172.08; 11 488.75]	14 840.00 [11 916.00; 15 866.00]	0.336	0.025	0.006	0.109
Gelsolin	6 487.90 [4 335.15; 8 448.57]	7 803.70 [7 184.55; 9 183.80]	6 671.10 [5 187.27; 8 562.55]	10 227.00 [7 514.25; 12 342.50]	0.101	0.028	0.215	0.219
Glutathione S-transferase P	1079.10 [654.89; 1476.33]	1 556.80 [1 259.60; 2 007.70]	1122.45 [747.97; 1500.75]	2 070.10 [1 522.95; 2 434.90]	0.035	0.006	0.608	0.156

Data are presented as median and interquartile range Me [Q1; Q3]. Values in bold indicate $p < 0.05$.

al cell adhesion molecule, coagulation factor V, fibrinogen α -chain, β -chain and γ -chain, tissue factor pathway inhibitor, von Willebrand factor (vWF)); chaperone proteins (heat shock protein β_1 , mitochondrial heat shock protein 60, GRP78 protein); transporter proteins (cation-independent mannose-6 phosphate receptor (CI-M6PR), phospholipid transfer protein). Significantly, most of the differentially represented proteins did not overlap between plasma and platelets. Thus, the platelet concentration of C8 β at the second stage of the study was higher in Group 1 than in Group 2, while the plasma concentration of this protein did not differ significantly.

We also analyzed the dynamics of the proteomic composition of blood plasma and platelets separately in each group (see Supplementary Materials on the journal's website: Tables S2-S5). In Group 1, the plasma concentrations of several proinflammatory proteins (C-reactive protein, serum amyloids A1 and A2, complement components C2, C4, C5, C8 β , C9, complement system subcomponent C1q B-chain, complement component C1r-like protein, haptoglobin, α_1 -acid glycoprotein 1), and hemostatic proteins (fibrinogen α -, β -, and γ -chains, α_2 -antiplasmin, vitronectin) were increased by stage 2 of the study. In Group 2, the concentrations of proinflammatory proteins (C-reactive protein, serum amyloids A1 and A2, complement components

Table 4 (end). Comparative analysis of platelet fraction proteins: the table shows proteins differentially represented between groups

Parameter	Values, fmol/μL				P			
	Stage 1		Stage 2		Group 1 vs. Group 2		Stage 1 vs. Stage 2	
	группа 1	группа 2	группа 1	группа 2	Stage 1	Stage 2	Group 1	Group 2
Heat shock protein β ₁	335.50 [228.78; 486.22]	587.89 [486.18; 609.18]	377.20 [252.24; 582.67]	795.42 [467.58; 845.12]	0.007	0.017	0.039	0.219
Metalloproteinase inhibitor 1	305.21 [164.25; 553.97]	548.12 [281.14; 591.69]	492.20 [309.30; 662.43]	915.97 [614.87; 1032.19]	0.162	0.012	<0.001	0.016
Metalloproteinase inhibitor 2	10.84 [6.94; 16.58]	15.13 [10.81; 20.10]	15.10 [11.01; 20.19]	21.38 [18.54; 27.07]	0.141	0.033	<0.001	0.047
Neuropilin 2	33.40 [26.06; 43.06]	47.06 [43.98; 53.20]	40.01 [30.11; 54.13]	70.35 [46.19; 82.79]	0.042	0.031	0.003	0.078
P-selectin	663.38 [413.17; 829.39]	756.43 [667.81; 1184.35]	642.20 [481.99; 895.72]	1269.40 [843.34; 1451.90]	0.096	0.028	0.142	0.109
Peroxisredoxin-1	642.20 [481.99; 895.72]	1269.40 [843.34; 1451.90]	512.22 [398.34; 633.39]	917.81 [774.35; 929.19]	0.028	0.005	0.330	0.156
Platelet endothelial cell adhesion molecule	608.08 [366.64; 830.69]	807.93 [690.10; 1191.75]	613.53 [452.88; 768.22]	1123.30 [883.58; 1339.15]	0.025	0.007	0.407	0.469
Deglycase DJ-1	1237.60 [762.74; 1575.22]	1523.30 [1263.65; 1822.35]	1214.40 [851.31; 1539.32]	1976.50 [1549.75; 2264.50]	0.087	0.011	0.407	0.375
Protein S100-A9	209.26 [44.70; 491.52]	45.98 [16.23; 59.40]	112.86 [26.23; 429.84]	22.15 [14.70; 28.42]	0.035	0.008	0.230	0.219
Secreted protein acidic and rich in cysteine	648.01 [330.30; 1149.15]	1128.50 [657.74; 1228.55]	1050.85 [688.53; 1345.07]	2072.00 [1322.00; 2331.40]	0.106	0.010	<0.001	0.016
Tissue factor pathway inhibitor	22.22 [14.81; 36.65]	32.340 [27.15; 46.97]	26.27 [19.58; 34.89]	61.87 [39.32; 66.73]	0.078	0.023	0.166	0.078
Von Willebrand factor	331.28 [219.07; 513.20]	432.97 [399.93; 674.10]	353.10 [221.84; 457.19]	683.59 [634.05; 757.45]	0.798	0.001	0.552	0.078
Mitochondrial heat shock protein 60	648.71 [427.20; 909.05]	915.12 [836.40; 1241.55]	645.36 [462.76; 854.34]	1417.10 [1037.32; 1431.40]	0.019	0.007	0.718	0.109
Protein GRP78	1143.35 [619.19; 1490.33]	1536.90 [1392.95; 2254.45]	1146.00 [846.81; 1496.77]	2190.40 [1765.40; 2409.00]	0.030	0.009	0.688	0.297

Data are presented as median and interquartile range Me [Q1; Q3]. Values in bold indicate p<0.05.

C4, C5, C8β, C9, complement component C1r-like protein, haptoglobin, α₁-acid glycoprotein 1) and hemostatic proteins (fibrinogen α-chain and γ-chain) were also increased. It should be particularly noted that in both groups, fibrinogen concentrations increased by the second stage of the study. This is consistent with the observed increase in clot density.

The dynamics of concentrations of platelet fraction proteins in group 1 showed increased concentrations of the proteins that regulate the immune system (C-reactive protein, serum amyloids A1 and A2, lipopolysaccharide-binding protein, leucine-rich α₂-glycoprotein, α₁-acid glycoprotein 1, vitronectin, attractin, ceruloplasmin, complement system components: subcomponent C1r, subcomponent C1q subunit C, subcomponents C1s, C3, C4, C5, C8β, C9, and factor B) and proteins of the hemostasis system (fibrinogen α-chain, β-chain and γ-chain, antithrombin III, plasminogen, prothrombin, coagulation factors X and XII, vitamin

K-dependent protein S). Group 2 also showed a dynamic increase in proinflammatory proteins (C-reactive protein, α₁-acid glycoprotein 1, haptoglobin, serum amyloids A1 and A2, complement component C1r-like protein, complement components C4, C5, C8β, C9) and hemostatic system proteins (fibrinogen α-chain and γ-chain).

Analysis of predictors for increased risk of thrombotic complications

Thus, we identified the proteins whose concentrations were differentially represented in the study groups. Using univariate regression analysis, we identified the proteins whose concentrations were associated with an increased risk of ischemic complications. Among these proteins, we determined the platelet fraction protein vWF, the prognostic value of whose plasma concentration has been described previously [18, 19]. In addition, platelet pro-

Каждый 5-й пациент перенесет повторный инфаркт миокарда в течение года после первого события¹.

Достижение целевого уровня ХС ЛНП — ключ к улучшению прогноза пациента. Пациент не узнает об этом без **вашей рекомендации**.



<1,4 ммоль/л
и **снижение $\geq 50\%$**
от исходного

Достижение целевого
уровня ХС ЛНП



8 (± 4) недель

Контроль липидного профиля –
через 8 (± 4) недель после начала
терапии и интенсификация
терапии при недостижении
целевого уровня ХС ЛНП

Если у пациента, перенесшего ИМ, при использовании максимально переносимой дозы статина в сочетании с эзетимибом концентрация ХС ЛНП в крови остаётся выше целевого уровня, **рекомендуется добавить инклисиран или ингибитор PCSK9** для дополнительного снижения уровня ХС ЛНП в крови и риска ишемических событий^{2,3}.



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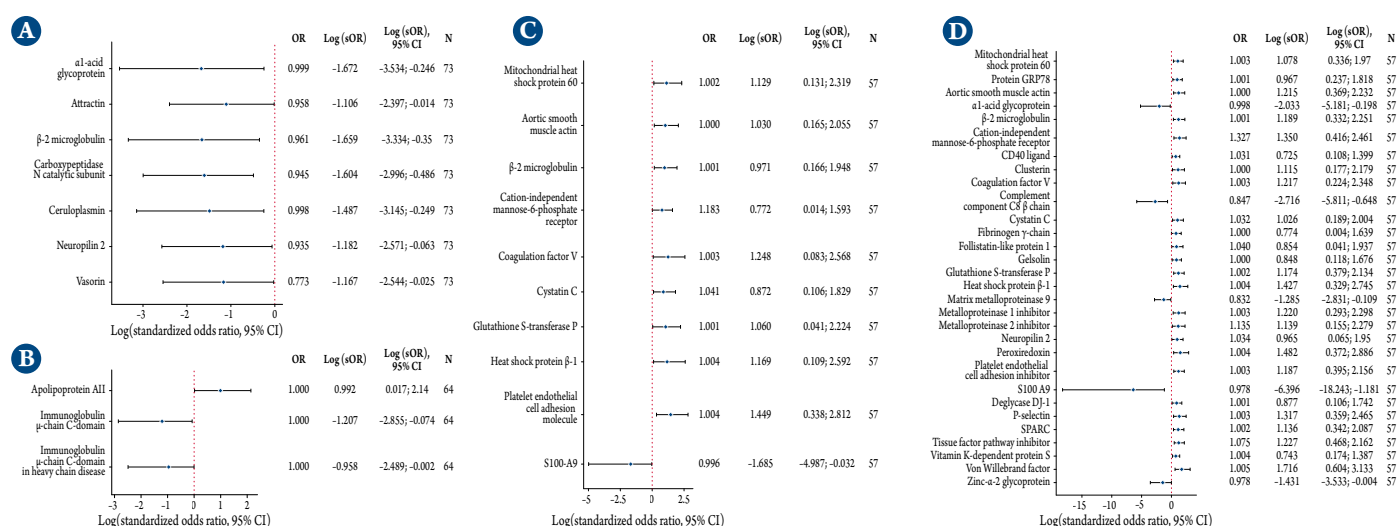
ХС ЛНП — холестерин липопротеинов низкой плотности; ИМ — инфаркт миокарда.

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ИНФОРМАЦИЯ ПРЕДНАЗНАЧЕНА ДЛЯ РАБОТНИКОВ ЗДРАВООХРАНЕНИЯ
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Figure 1. Characteristics of the relationship between protein concentrations in blood plasma and platelet fraction with the likelihood of ischemic complications outside the hospital: Results of univariate regression analysis



The figure shows proteins, whose concentration demonstrated a statistically significant association with the development of ischemic complications: A, blood plasma proteins (study stage 1); B, blood plasma proteins (study stage 2); C, platelet fraction proteins (study stage 1); D, platelet fraction proteins (study stage 2).

teins C8β and CI-M6PR had some of the highest risk ratio values (see Fig. 1).

Discussion

In this study, we analyzed a wide range of hemostasis parameters and assessed the proteomic composition of blood plasma and platelets in patients with AMI associated with DAPT. The study patients were divided into 2 groups: patients without thrombotic complications during the follow-up period (group 1) and patients with thrombotic complications (group 2). According to aggregometry data obtained from the Multiplate analyzer, platelet aggregation values upon stimulation with arachidonic acid or ADP in both groups reached the target values during antiplatelet therapy. We searched for factors associated with the increased risk of thrombotic complications among plasma proteins and in the platelet fraction. We assumed that platelet proteins would be the most sensitive markers of a high risk for ischemic complications. Indeed, according to our data, differentially represented platelet proteins had a greater prognostic value for the risk of thrombotic complications compared to plasma proteins. Among platelet proteins, vWF should be particularly noted. The vWF is associated with the risk of thrombotic complications in patients with myocardial infarction and multifocal atherosclerosis [19–21]. The vWF is a protein that plays a key role in platelet adhesion [18]. There are limited data on the functional and clinical significance of the platelet fraction vWF [22]. Platelet vWF is known to be present in platelet α-granules and secreted locally upon their activation [23]. Since platelet vWF is secreted locally,

it is less susceptible to ADAMTS-13 cleavage and retains the structure of ultra-large multimers that are known to have a greater activity in the adhesion process than plasma multimers [23, 24]. In a laboratory model of ischemic stroke, platelet vWF did not affect hemostasis in case of vascular wall damage, but played a role in atherothrombosis [25]. We demonstrated for the first time the association of platelet vWF with an unfavorable prognosis associated with the DAPT treatment in AMI patients.

The analysis of these parameters in dynamics revealed that the platelet concentration of vWF, like that of several other proteins, increased at 3–5 days after PCI and the administration of DAPT. Since the activity of platelet protein synthesis is low, it is most likely that the vWF accumulation occurs through its binding to platelet surface receptors. The ability of platelets to internalize molecules has also been reported, which may be a second potential mechanism for increasing protein concentrations in platelets [26, 27]. Further study is required to establish the precise mechanisms for the dynamics of vWF concentration in platelets.

Another protein with the highest prognostic significance for thrombotic complications in AMI patients was the C8β protein in the blood platelet fraction. According to our data, an increase in the C8β concentration is associated with a reduced risk of thrombotic complications in these patients. C8β is involved in the formation of membrane attack complex [28]. Inhibition of the membrane attack complex formation can contribute to a decrease in platelet activation, which is consistent with our results [29]. Also, the complement system has been reported to

play a role in the post-infarction impairment of myocardial reperfusion [30]. However, the role of C8 β in atherothrombosis is understudied and requires further investigation [31].

The third promising predictor of thrombotic complications associated with DAPT is the platelet protein CI-M6PR. This protein is involved in the transport of lysosomal enzymes, including cathepsins [32]. CI-M6PR localized on the platelet membrane can bind heparanase, an enzyme that degrades the extracellular matrix component, heparan sulfate [33]. However, the functional significance of this protein in platelets remains unclear.

Our hemostasis study demonstrated for the first time that the thrombus density increased in both AMI groups after PCI and initiation of DAPT. Considering that the increased thrombus density was observed in selective assessment of the hemostasis plasma component by the Thrombodynamics method, while the functional activity of the platelet component only decreased over time, it seems most likely that the increase in thrombus density was due to the activity of soluble coagulation factors. Fibrinogen is the key factor in the formation of thrombus density [34]. According to our data, the fibrinogen concentration (both in plasma and platelets) also increased by stage 2 in both Group 1 and Group 2, which confirmed the above hypothesis.

Also, it has been reported that fibrinogen is an acute phase protein of inflammation and is involved in the regulation of inflammatory process activity [35]. We observed dynamic increases in concentrations of proinflammatory markers in both groups, indicative of activation of the inflammatory process by day 3–5 after PCI, which is consistent with published reports [36]. It is worth noting that, due to the small size of Group 2, our major conclusions about the dynamics of plasma and platelet protein concentrations and the values of hemostasis functional activity parameters, were based on the analysis of Group 1. The elevated inflammatory activity may be one of the reasons for the increase in plasma fibrinogen concentration and,

thus, for the increase in thrombus density. The increasing activity of endogenous lysis over time may occur as a consequence of the increasing fibrinogen concentration and thrombus density. Patients in Group 2 also had dynamically increasing clot lysis time that was not observed in the group without ischemic complications. Differences in the dynamics of fibrinogen concentration and endogenous lysis activity can also be used in developing a new approach to detection of an increased risk of ischemic complications associated with the DAPT in AMI patients.

Study limitations

The main limitation of this study is the relatively small sample size. Due to the low incidence of ischemic complications outside the hospital, the distribution of patients across the groups was uneven. The relatively low incidence of ischemic complications was due to the study design, which excluded patients with very high-risk AMI (complicated AMI).

Conclusion

In this study, we identified the proteins, elevations or decreases in which concentrations were associated with an increased risk of thrombotic complications in patients with acute myocardial infarction receiving dual antiplatelet therapy. The most promising protein markers we identified were the platelet proteins: von Willebrand factor, C8 β , and CI-M6PR. An additional randomized prospective study is required before implementing these results in clinical practice.

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SUPPLEMENTARY MATERIALS

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