

# Platelets characteristics in patients with coronary artery disease and atrial fibrillation

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coronary artery disease, atrial fibrillation, amino acids, blood platelets.

## Ключові слова:

ішемічна хвороба серця, фібриляція передсердь, амінокислоти, тромбоцити.

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**The aim.** To estimate the connections between platelet morphological characteristics and amino acids (AA) profile in patients with coronary artery disease (CAD) and atrial fibrillation (AF).

**Materials and methods.** 300 patients were included in the study. They were divided into 3 groups: first (CAD) – 149 patients with CAD but without arrhythmias, second (CAD+AF) – 124 patients with CAD and AF paroxysm and control group – 27 patients without CAD and arrhythmias. Platelets AA level was detected by method of ion exchange liquid column chromatography. Total platelet count (PC), mean platelet volume (MPV), platelets distribution width (PDW), platelet-to-leukocyte (PLR) were obtained from common blood count.

**Results.** Significant decline of PC and rise of MPV and PLR ratio was found in CAD patients and AF paroxysm in comparison with group with CAD without arrhythmia,  $p < 0.05$ . Significant increase of isoleucine, leucine and decrease of threonine, serine, glycine, valine levels was found, changes in branched chain amino acids (rise of Isoleucine + Leucine / Valine ratio) and Glycine + Serine (decline Glycine + Serine sum) metabolism were revealed in CAD patients and AF paroxysm in comparison with group with CAD without arrhythmia,  $p < 0.05$ . Phenylalanine / Tyrosine ratio was significantly elevated in CAD patients with and without AF,  $p < 0.05$ .

Taurine, serine, and glycine correlated with platelets morphological characteristics: PC ( $r = 0.714$ ,  $r = 0.732$  and  $r = 0.340$  consequently), MPV ( $r = -0.724$ ,  $r = -0.390$  and  $r = -0.571$  consequently), PDW ( $r = -0.666$  and  $r = -0.364$  consequently) and PLR ( $r = 0.586$  and  $r = 0.648$  consequently),  $p < 0.05$ . Platelets glycine (AUC = 0.8760), valine (AUC = 0.8707), leucine (AUC = 0.8267) and threonine (AUC = 0.8213) levels are closely connected with AF paroxysm in CAD patients by ROC-analysis results,  $p < 0.05$ .

Platelets amino acids combinations: Glycine + 0.6 × Valine (AUC = 0.9987), Threonine – 3.6 × Leucine (AUC = 0.9040) were found in prediction AF paroxysm for CAD patients by ROC-analysis results,  $p < 0.05$ .

**Conclusions.** Connections between platelets morphology and AA profile violations in patients with CAD and AF paroxysm were found in our study.

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## Характеристики тромбоцитів у пацієнтів з ішемічною хворобою серця та фібриляцією передсердь

I. O. Мельничук

**Мета роботи** – оцінити зв'язки між морфологічними характеристиками та амінокислотним (АК) профілем тромбоцитів у хворих на ішемічну хворобу серця (ІХС) із фібриляцією передсердь (ФП).

**Матеріали та методи.** У дослідження залучили 300 пацієнтів, яких поділили на три групи: перша (ІХС) – 149 хворих на ІХС без аритмій; друга (ІХС + ФП) – 124 обстежених з ІХС і пароксизмом ФП; контрольна група – 27 осіб без ІХС та аритмій. Рівень АК тромбоцитів визначали методом іонообмінної рідинної колонкової хроматографії. Загальну кількість тромбоцитів (РС), середній об'єм тромбоцитів (МРВ), ширину розподілу тромбоцитів (РДВ), співвідношення тромбоцитів та лейкоцитів (ПЛР) визначили під час загального аналізу крові.

**Результати.** У пацієнтів з ІХС і пароксизмом ФП виявлено достовірне зниження РС і підвищення МРВ і ПЛР порівняно з групою з ІХС і без аритмії ( $p < 0,05$ ). У пацієнтів з ІХС і пароксизмом ФП порівняно з групою хворих на ІХС без аритмії встановили значне підвищення рівнів ізолейцину, лейцину та зниження рівнів треоніну, серину, гліцину, валіну; зафіксовано зміни метаболізму амінокислот із розгалуженим ланцюгом (підвищення співвідношення ізолейцин + лейцин / валін) і гліцин + серин (зменшення суми гліцин + серин),  $p < 0,05$ . Співвідношення фенілаланін / тирозин істотно підвищене в пацієнтів з ІХС з ФП та без неї,  $p < 0,05$ .

Таурин, серин і гліцин корелюють із морфологічними характеристиками тромбоцитів: РС ( $r = 0,714$ ,  $r = 0,732$  і  $r = 0,340$  відповідно), МРВ ( $r = -0,724$ ,  $r = -0,390$  і  $r = -0,571$  відповідно), РДВ ( $r = -0,666$  і  $r = -0,364$  відповідно) і ПЛР ( $r = 0,586$  і  $r = 0,648$  відповідно),  $p < 0,05$ . За результатами ROC-аналізу, рівні гліцину (AUC = 0,8760), валіну (AUC = 0,8707), лейцину (AUC = 0,8267) і треоніну (AUC = 0,8213) тромбоцитів тісно пов'язані з пароксизмом ФП у пацієнтів з ІХС,  $p < 0,05$ .

У результаті ROC-аналізу комбінації амінокислот тромбоцитів, а саме гліцин + 0,6 × валін (AUC = 0,9987), треонін – 3,6 × лейцин (AUC = 0,9040), запропоновані для прогнозу пароксизму ФП у пацієнтів з ІХС,  $p < 0,05$ .

**Висновки.** Визначили зв'язки між змінами морфології та амінокислотного профілю тромбоцитів у пацієнтів з ІХС і пароксизмом ФП.

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Atrial fibrillation (AF) is the most common cardiac arrhythmia in adults, and it is associated with significant morbidity and mortality. Coronary artery disease (CAD) is a known AF risk factor. CAD and AF have most mutual risk factors and can worsen clinical picture and prognosis. Stroke is one of the most common AF complications. Ischemic stroke is a prevalent among AF patients. As well as thromboembolism is a common AF complication. Therefore, anticoagulant therapy is the basis in AF treatment [1,2].

Mechanisms of increased platelets activity in CAD patients are well known. But increased prothrombotic tendency pathogenesis in AF patients is still under study. AF is associated with platelets activation. Activated platelets have a lot of prothrombotic and vasoactive properties. Hemostatic balance is represented by platelets condition and its violations leads for supraventricular arrhythmias development [3]. Moreover, platelet-derived biomarkers are an attractive target for secondary prophylaxis in CAD patients, because CAD pathogenesis is directly related to thrombosis occurrence [4].

Recent studies have reported about association of platelets characteristics with AF occurrence. AF is characterized by decreasing platelets count (PC), increasing mean platelet volume (MPV) and platelets distribution width (PDW). MPV is the reliable marker of platelets activation [3]. Branched chain amino acids (BCAA) metabolism is deeply implicated in platelets activation in animal studies. Valine /  $\alpha$ -ketoisovaleric acid catabolic pathway switching-on can facilitated platelets activation by increase of integrin expression  $\alpha$ IIb $\beta$ 3 and propionylation of tropomodulin-3 in platelets [5].

Plasma BCAA levels are directly connected with arterial thrombosis. Also, circulating BCAA positively correlates with cardiovascular risks, arterial hypertension, and insulin resistance. In vivo BCAA are capable to increase agonist-induced activation, granule release, aggregation and spreading of platelets [6].

On the other hand, glycine can reduce platelets activation by influencing glycine-gated chlorine channels in platelets [7]. In vitro taurine is also applicable to reduce platelets activations by tissue-type plasminogen activator and extent platelets shape exchange [8].

The role of platelets characteristics in the AF pathogenesis and AF paroxysm prediction remains completely unclear nowadays. Moreover, there is not enough studies about platelets AA profile in AF and CAD patients. Most data on the amino acids role in platelets activation are obtained from animal studies and in vivo experiments.

## Aim

To estimate the connections between platelet morphological characteristics and amino acids profile in patients with coronary artery disease and atrial fibrillation.

## Materials and methods

We examined 300 patients and divided them into 3 groups: first (I) – 149 patients with CAD and without arrhythmias, second (II) – 123 patients with CAD and AF paroxysm and control group (CG) – 28 patients without CAD and arrhythmias. CAD and AF diagnosis were based on latest ESC guidelines [1,2]. CAD diagnosis was confirmed by history of coronary arteries stenotic changes at invasive coronarography. AF paroxysm was checked by resting 12 leads electrocardiography (ECG).

Exclusion criteria were registered malignancies, chronic kidney disease (Glomerular Filtration Rate, GFR <60 mL/min), valvular AF, heart failure Class III to IV (by New York Heart Association), thyroid pathology, inflammatory bowel disease, irritable bowel syndrome, vegetarians and vegans, pregnancy, taking probiotics and antibiotics for a month before the study. There were no significant differences in risk factors at baseline between the study groups.

The study was based on and approved by the ethic commission of the Kyiv City Clinical Hospital No. 12. Informed consent was obtained from all subjects in accordance with the Declaration of Helsinki.

Baseline characteristics of study sample are shown in *Table 1*.

Characteristics such as PC, MPV, PDW, platelet-to-leukocyte ratio (PLR) and platelets AA profile were investigated. PC, MPV, PDW, PLR were obtained from common blood count. Platelets AA level was detected by method of ion exchange liquid column chromatography – such AA were identified: lysine, histidine, arginine, ornithine, taurine, asparagine acid, threonine, serine, glutamine acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, glutamine, ammonia.

Blood sampling from patients was performed on an empty stomach from the cubital vein on the first day of hospitalization, before treatment. Citrated blood was centrifuged for 10 minutes at a speed of 1500 revolutions per minute. The middle layer was selected with a Pasteur pipette – the plasma is saturated with platelets. The obtained material was again centrifuged for 20 minutes at a speed of 3000 revolutions per minute. The upper supernatant liquid was collected with a Pasteur pipette, and the lower layer was washed with buffer (pH 6.2). Washed platelets were resuspended in buffer (pH 7.4).

Results were presented as mean  $\pm$  standard error or [95 % confidence interval (CI)] for continuous variables or as a number for categorical variables. Data were compared using Wilcoxon signed-rank test or Student t-test with two critical regions by the type of distribution; Spearman's rank correlation coefficient. ROC-curve analysis for lipid and inflammatory indexes and linear regression model were done. Area under ROC-curve (AUC) was calculated [8,9,10]. All calculations were done in MATLAB R2014a (License number 271828).

**Table 1.** Baseline characteristics of study sample, mean  $\pm$  standard error

Parameter, units of measurement	CAD	CAD + AF	CG	p1–2	p2–3	p1–3
Age, years	67.71 $\pm$ 3.90	67.96 $\pm$ 0.94	56.25 $\pm$ 2.18	>0.05	>0.05	>0.05
Men, %	48.99	47.97	48.15	>0.05	>0.05	>0.05
BMI, kg/m <sup>2</sup>	27.02 $\pm$ 0.33	26.93 $\pm$ 0.43	28.12 $\pm$ 2.10	>0.05	>0.05	>0.05
Uric acid, mmol/L	380.5 $\pm$ 28.16	404.90 $\pm$ 36.11	310.20 $\pm$ 29.12	>0.05	<0.05	<0.05
Total bilirubin, mmol/L	11.30 $\pm$ 0.09	12.40 $\pm$ 0.08	11.70 $\pm$ 0.11	>0.05	>0.05	>0.05
GFR, ml/min	62.03 $\pm$ 2.31	67.73 $\pm$ 1.98	84.01 $\pm$ 5.48	>0.05	<0.05	<0.05
Total cholesterol, mmol/L	5.73 $\pm$ 0.37	6.18 $\pm$ 0.31	4.32 $\pm$ 0.21	>0.05	<0.05	<0.05

**Table 2.** Platelets characteristics in investigated groups, mean  $\pm$  standard error

Parameter	CAD	CAD + AF	CG	p1–2	p2–3	p1–3
PC	240.20 $\pm$ 7.80	210.90 $\pm$ 5.78	305.00 $\pm$ 7.79	<0.05	<0.05	<0.05
MPV	10.86 $\pm$ 0.09	11.87 $\pm$ 0.08	8.13 $\pm$ 0.14	<0.05	<0.05	<0.05
PDW	12.38 $\pm$ 0.17	12.50 $\pm$ 0.14	10.39 $\pm$ 0.20	>0.05	<0.05	<0.05
PLR	34.07 $\pm$ 1.23	48.08 $\pm$ 3.19	30.32 $\pm$ 1.01	<0.05	<0.05	<0.05

## Results

Platelets morphological sings were investigated in the studied groups in our study. In the first group significant decrease in PC (21.25 %) and increase in MPV (33.58 %), PDW (20.31 %) and PLR (12.37 %) compared to CG were found. Also, in the second group significant decrease in PC (30.85 %) and increase in MPV (46.00 %), PDW (20.31 %) and PLR (58.58 %) compared to CG were obtained. Moreover, in the second group significant decrease in PC (12.20 %) and increase in MPV (9.30 %) and PLR (41.12 %) compared to the first group were observed.

Results are shown in *Table 2*.

Also, platelets amino acids profile was detected in studied groups in our investigation. In the first group significant increase in isoleucine (12.41 %) and decrease in taurine (20.26 %), serine (9.31 %) and glycine (19.73 %) levels were found in comparison with CG. In the second group we observed significant rise of isoleucine (24.47 %), leucine (10.20 %) and depletion of taurine (19.84 %), threonine (29.37 %), serine (13.90 %), glycine (45.59 %) and valine (27.87 %) levels in comparison with CG. Furthermore, in the second group significant increase of isoleucine (10.73 %), leucine (12.63 %) and decrease of threonine (23.05 %), serine (5.06 %), glycine (32.21 %), valine (30.83 %) levels were found in comparison with the first group.

Results are shown in *Table 3*.

Different AA combinations were compared for deeper understanding their pathogenetic role. We have detected: branched chain AA (BCAA) – Isoleucine + Leucine / Valine ratio, aromatic amino acids (AAA) – Phenylalanine/ Tyrosine ratio, Glycine + Serine sum. Significant increase of Phenylalanine / Tyrosine ratio (10.34 %) and decrease of Glycine + Serine sum (15.73 %) was found in the first group compared to CG. Also, significant increase of Isoleucine + Leucine / Valine ratio (55.28 %) and decrease of

Glycine + Serine sum (33.37 %) was found in the second group compared to CG. Moreover, significant rise of Isoleucine + Leucine / Valine ratio (61.19 %) and depletion of Glycine + Serine sum (20.93 %) was revealed in the second group compared to the first one.

All results are shown in *Table 4*.

Correlation analysis between morphological characteristics of platelets and AA profile in the studied groups was performed. The largest number of correlations was observed between platelets AA profile and their morphological characteristics as PC (total number = 5), PLR (total number = 5) and MPV (total number = 6) levels. On the other hand, the highest number of correlations was found between taurine (total amount = 3), serine (total number = 3) and platelets morphological characteristics.

All correlations are shown in the *Fig. 1*.

ROC-analysis was performed individually for each platelet AA, for further validation of their diagnostic values AUC was established for each platelet AA. Seven acceptable results were found in our study: AUC more then 0.7 was detected for threonine, serine, glutamine acid, glycine, valine, isoleucine, and leucine – it is concern as acceptable result [11].

Results are shown in *Table 5*.

AUC more then 0.8 was found for threonine, glycine, valine, and leucine – we consider this as good result [11].

The best four of obtained ROC-curves are shown in *Fig. 2*.

Also, we tried to find the optimal platelets AA combination with the highest AUC through results of the first and second groups.

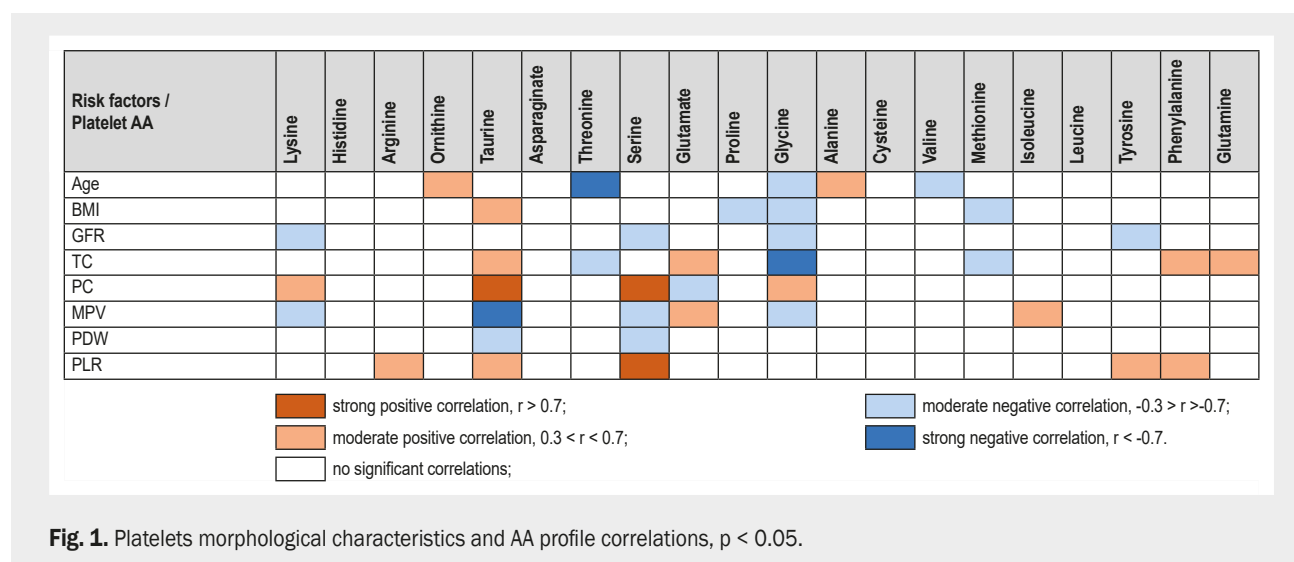
We got two excellent (AUC more then 0.9) [11] combinations: Glycine + 0.6  $\times$  Valine, AUC = 0.9987,  $p < 0.05$ ; Threonine – 3.6  $\times$  Leucine, AUC = 0.9040,  $p < 0.05$ . ROC-curves are shown in *Fig. 3*.

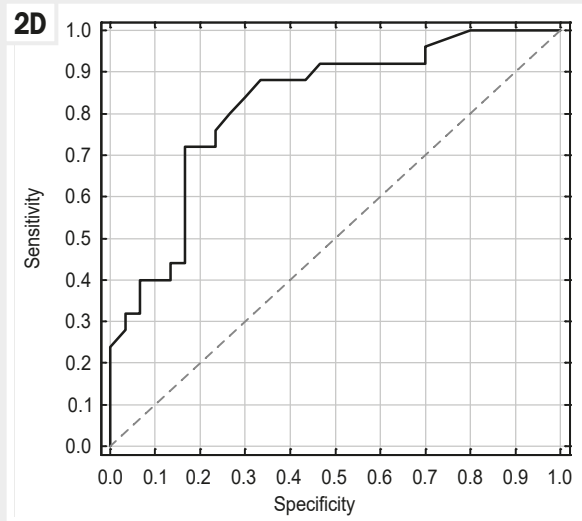
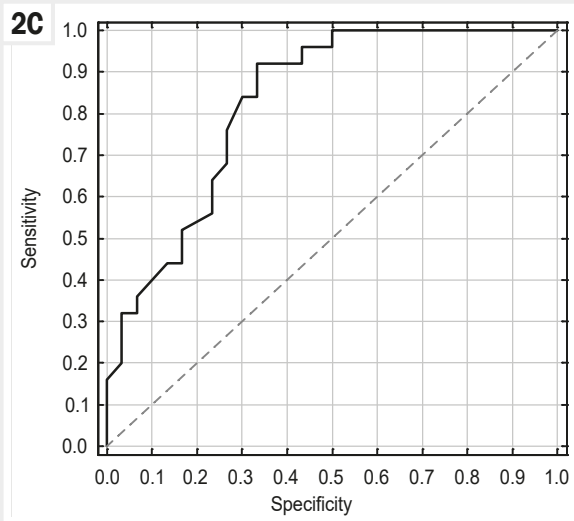
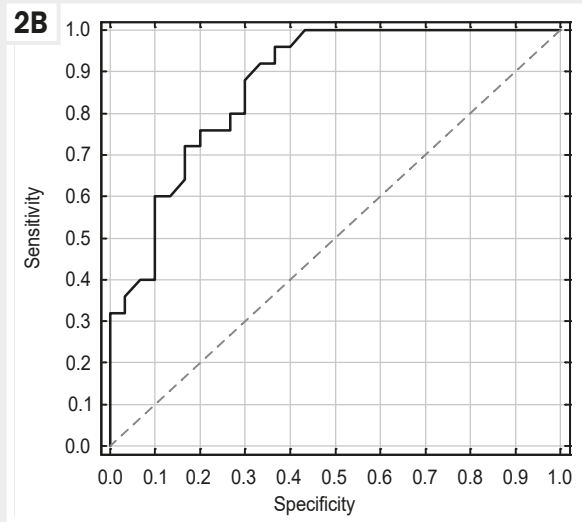
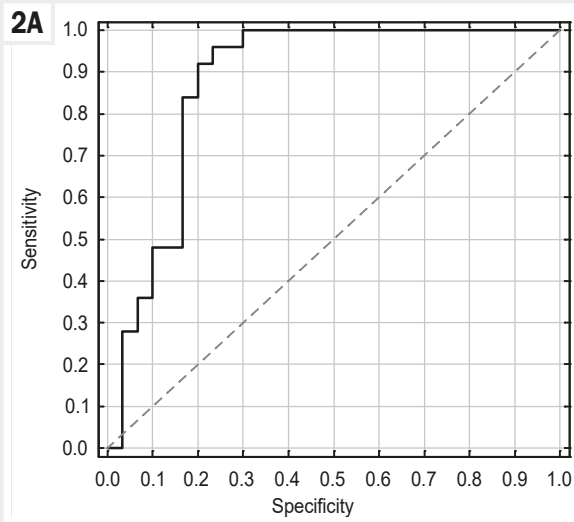
**Table 3.** Platelets AA spectrum in investigated groups, mean ± standard error, mkmol/L

Parameter	CAD	CAD + AF	CG	p1-2	p2-3	p1-3
Lysine	100.30 ± 3.81	98.27 ± 3.05	105.60 ± 5.89	>0.05	>0.05	>0.05
Histidine	191.80 ± 5.37	194.20 ± 4.84	194.20 ± 11.26	>0.05	>0.05	>0.05
Arginine	103.50 ± 3.12	102.80 ± 1.86	102.90 ± 7.11	>0.05	>0.05	>0.05
Ornithine	59.64 ± 1.51	58.37 ± 1.82	57.10 ± 3.19	>0.05	>0.05	>0.05
Taurine	3066.00 ± 20.58	3082.00 ± 17.18	3845.00 ± 74.49	>0.05	<0.05	<0.05
Asparagine acid	237.80 ± 4.20	228.90 ± 1.99	234.30 ± 16.21	>0.05	>0.05	>0.05
Threonine	184.40 ± 7.18	141.90 ± 5.53	200.90 ± 7.62	<0.05	<0.05	>0.05
Serine	205.60 ± 2.16	195.20 ± 2.64	226.70 ± 6.35	<0.05	<0.05	<0.05
Glutamine acid	428.00 ± 5.46	415.80 ± 26.13	394.70 ± 18.90	>0.05	>0.05	>0.05
Proline	73.44 ± 1.59	70.90 ± 1.58	68.10 ± 6.18	>0.05	>0.05	>0.05
Glycine	290.00 ± 3.59	196.60 ± 2.19	361.30 ± 17.27	<0.05	<0.05	<0.05
Alanine	375.20 ± 2.76	369.80 ± 3.82	383.90 ± 5.85	>0.05	>0.05	>0.05
Cysteine	88.60 ± 1.71	81.57 ± 1.32	80.80 ± 7.69	>0.05	>0.05	>0.05
Valine	170.60 ± 2.24	118.00 ± 2.12	163.60 ± 11.88	<0.05	<0.05	>0.05
Methionine	75.12 ± 1.11	73.60 ± 0.93	80.40 ± 7.14	>0.05	>0.05	>0.05
Isoleucine	512.70 ± 4.34	567.70 ± 12.55	456.10 ± 16.63	<0.05	<0.05	<0.05
Leucine	199.60 ± 2.28	224.80 ± 4.58	204.00 ± 7.11	<0.05	<0.05	>0.05
Tyrosine	135.30 ± 1.43	147.30 ± 4.18	137.20 ± 8.11	>0.05	>0.05	>0.05
Phenylalanine	86.36 ± 2.24	82.53 ± 1.46	83.90 ± 3.38	>0.05	>0.05	>0.05
Glutamine	306.60 ± 2.90	308.90 ± 4.28	314.70 ± 8.35	>0.05	>0.05	>0.05

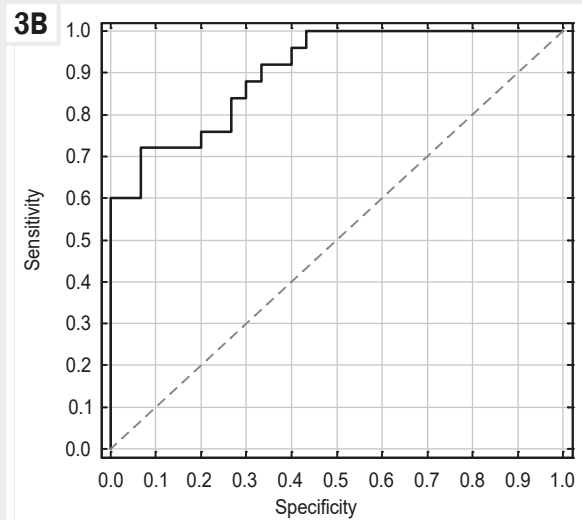
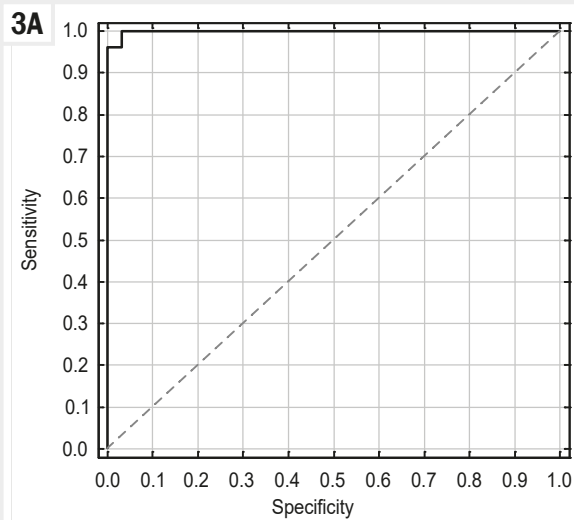
**Table 4.** Plasma AA spectrum combinations in CAD patient with or without AF compared to control group, mean [95 % CI], mkmol/L

Parameter	CAD	CAD + AF	CG	p1-2	p2-3	p1-3
Isoleucine + Leucine / Valine	4.20 ± 0.05	6.77 ± 0.15	4.36 ± 0.52	<0.05	<0.05	>0.05
Phenylalanine/ Tyrosine	0.64 ± 0.02	0.63 ± 0.05	0.58 ± 0.02	>0.05	>0.05	<0.05
Glycine + Serine	495.50 ± 2.82	391.80 ± 3.71	588.00 ± 18.45	<0.05	<0.05	<0.05





**Fig. 2.** ROC-analysis of platelets AA in the first and second groups,  $p < 0.05$ . **A:** Glycine, AUC = 0.8760; **B:** Valine, AUC = 0.8707; **C:** Leucine, AUC = 0.8267; **D:** Threonine, AUC = 0.8213.



**Fig. 3.** ROC-analysis of platelets AA combinations in the first and second groups,  $p < 0.05$ . **A:** Glycine +  $0.6 \times$  Valine, AUC = 0.9987; **B:** Threonine -  $3.6 \times$  Leucine, AUC = 0.9040.

**Table 5.** ROC-curve analysis – area under ROC curve for each platelet AA in first and second groups,  $p < 0.05$ 

Platelets AA	AUC
Lysine	0.5313
Histidine	0.5360
Arginine	0.5013
Ornithine	0.5513
Taurine	0.6480
Asparagine acid	0.6180
Threonine	<b>0.8213</b>
Serine	<b>0.7080</b>
Glutamine acid	<b>0.7307</b>
Proline	0.5920
Glycine	<b>0.8760</b>
Alanine	0.5767
Cysteine	0.7353
Valine	<b>0.8707</b>
Methionine	0.5693
Isoleucine	<b>0.7527</b>
Leucine	<b>0.8267</b>
Tyrosine	0.6700
Phenylalanine	0.6727
Glutamine	0.5147

## Discussion

Platelets morphological changes during AF paroxysm in CAD patients were found in our study. They included decline of total PC and rise of MPV and PLR. These changes were predictable according to the recent literature data [3,4]. AF paroxysm pathogenesis is based on thrombosis and inflammation processes [3]. Platelet's role in coagulation now is already have proved. It is based on release of variety biological active factors from platelets granules during their activation. They include thrombin, adenosine diphosphate, thromboxane A<sub>2</sub>, and the phosphorylation of various target proteins, resulting for high-affinity ligands binding and platelets aggregation. Moreover, platelets  $\alpha$ -granules are composed of adhesion proteins (fibrinogen, fibronectin, thrombospondin, vitronectin, von Willebrand factor), chemokines (Platelet factor 4,  $\beta$ -Thromboglobulin, Macrophage inflammatory protein 1 $\alpha$ , etc.), coagulation proteins (factors V, VII, XI, XIII, kininogens, plasminogen, protein S) and growth factors (Angiopoietin-1 and 2, Endothelial cell growth factor, Interleukin-2, 3, 4, 6, 7, and 8, Interleukin- $\beta$ , etc.), immunoglobulins, protease inhibitors, proteoglycans, etc. [12]. MPV is an important marker of platelets activity. Secretion from platelets granules is characteristic of large cells. By the latest data, MPV is correlated with major cardiovascular risk factors as hypercholesterolemia, dyslipidemia, hyperglycemia, etc. Increased MPV is closely associated with CAD and its compli-

cations. Thus, it can be used as a marker of the risk stratification in CAD patients. PDW is also known, but less sensitive factor of platelets activity. It is also increased for CAD and AF patients, but it also depends on different additional factors [3].

According to our data, PDW was not different in CAD and CAD with AF paroxysm groups. PLR is the possible marker of balance between thrombosis and inflammation: increased PLR can indicate the rise of risk platelets-rich thrombi formation. Also, it promotes inflammatory changes because increased PLR is associated with enhanced megakaryocytes proliferation [4]. However, connections between morphological and biochemical platelets structural changes have not yet been explored.

Platelets amino acids spectrum was investigated in our study: increase of isoleucine, leucine and decrease of threonine, serine, glycine, valine levels was found. Also, significant changes in BCAA (rise of Isoleucine + Leucine / Valine ratio) and Glycine + Serine metabolism were revealed in patients with CAD and AF in comparison with CAD patients without arrhythmias. BCAA metabolism violations directly takes part in thrombosis formation. Excessive plasma BCAA levels correlate closely with thrombosis. Isoleucine and leucine are metabolized in acetate and propionate consequently, valine is metabolized into butyrate. Valine /  $\alpha$ -ketoisovaleric acid catabolic pathway has the strongest promotion effect on platelets activation [5]. Moreover, BCAA promote thrombopoiesis by activating mTOR signaling pathway and increasing tropomodulin-3 propionylation [5,13].

Glycine is able to hyperpolarize cellular membrane by increasing chloride conduction. Glycine binds anion channel receptor and chlorine influx increases. It is known that in hyperpolarized cell calcium channel are more difficult to open. So, glycine inhibits platelets aggregation by minimizing of intracellular calcium growth [7]. Serine / threonine protein kinases plays the crucial role in platelets membrane receptors stimulation and subsequent second messenger signaling. Their inhibition blocks multiple intraplatelet and membrane signaling pathways and lead for platelets activation [14]. Phenylalanine / Tyrosine ratio was significantly elevated in CAD patients with and without AF. Tyrosine protein kinases and phosphor-tyrosine signaling pathway also directly regulate platelets activation [15]. Taurine and serine had the largest number of correlations with platelets morphological characteristics in our study. Taurine has known anti-inflammatory and antilipidemic properties. Also, taurine inhibits platelet-derived growth factor BB, that can decline platelets activation [8].

Moreover, in our study we provided ROC-analysis, that showed an improved role of glycine, valine, leucine, and threonine metabolism in AF paroxysm pathogenesis in CAD patients.

## Conclusions

Connections between platelets morphology and amino acids profile violations in patients with coronary artery disease and atrial fibrillation paroxysm were found in our study:

1. Significant decline of total platelet count and rise of mean platelet volume and platelet-to-leukocyte ratio was found in coronary artery disease patients and atrial fibrillation paroxysm in comparison to group with coronary artery disease and without arrhythmia,  $p < 0.05$ ;

2. Significant increase of isoleucine, leucine and decrease of threonine, serine, glycine, valine levels were found, changes in branched chain amino acids (rise of Isoleucine + Leucine / Valine ratio) and Glycine + Serine (decline Glycine + Serine sum) metabolism were revealed in coronary artery disease patients and atrial fibrillation paroxysm in comparison with group with coronary artery disease and without arrhythmia,  $p < 0.05$ ;

3. Phenylalanine / Tyrosine ratio was significantly elevated in coronary artery disease patients with and without atrial fibrillation,  $p < 0.05$ ;

4. Taurine, serine and glycine correlated with platelets morphological characteristics: total platelets count ( $r = 0.714$ ,  $r = 0.732$  and  $r = 0.340$  consequently), mean platelet volume ( $r = -0.724$ ,  $r = -0.390$  and  $r = -0.571$  consequently), platelets distribution width ( $r = -0.666$  and  $r = -0.364$  consequently) and platelet-to-leukocyte ratio ( $r = 0.586$  and  $r = 0.648$  consequently),  $p < 0.05$ ;

5. Platelets glycine (AUC = 0.8760), valine (AUC = 0.8707), leucine (AUC = 0.8267) and threonine (AUC = 0.8213) levels are closely connected with atrial fibrillation paroxysm in coronary artery disease patients by ROC-analysis results,  $p < 0.05$ ;

6. Platelets amino acids combinations: Glycine +  $0.6 \times$  Valine (AUC = 0.9987), Threonine –  $3.6 \times$  Leucine (AUC = 0.9040) were found for prediction of atrial fibrillation paroxysm in coronary artery disease patients according to ROC-analysis results,  $p < 0.05$ .

**Prospects for further research.** Platelets amino acids profile correction will be interesting approach for further investigations.

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