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PLASMA AMINO ACIDS SPECTRUM AS AN IMPORTANT PART OF METABOLOMIC PATTERN IN PATIENTS WITH CORONARY ARTERY DISEASE AND ATRIAL FIBRILLATION

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Ключові слова: *ішемічна хвороба серця, фібриляція передсердь, метаболіти мікробіому кишківника, амінокислоти плазми*

Abstract. Plasma amino acids spectrum as an important part of metabolomic pattern in patients with coronary artery disease and atrial fibrillation. Melnychuk I.O., Lyzogub V.H. *The aim of our work was to estimate the plasma amino acid (AA) spectrum peculiarities in coronary artery disease (CAD) patients with atrial fibrillation (AF) and to check their connections with cardiometabolic risk factors and gu, microbiota metabolites. 300 patients were divided into three groups: first – 149 patients with CAD but without arrhythmias, second – 123 patients with CAD and AF paroxysm and control group – 28 patients without CAD and arrhythmias. Plasma AA level was detected by ion exchange liquid column chromatography method. The plasma AA spectrum changes in CAD patients with AF paroxysm were investigated: some plasma AA (glutamate, glutamine, glycine, alanine, valine, tyrosine) and their combinations (Isoleucine+Leucine/Valine, Glycine+Serine, Glycine/Methionine, Phenylalanine/Tyrosine, Glutamine/Glutamate) had significant changes in second group patients and had correlations with cardiometabolic risk factors (glycine, valine, arginine, glutamate, isoleucine, alanine, methionine ($p<0.05$)). Plasma AA combinations were revealed, the lattert could be used as an early marker of AF paroxysm in CAD patients by the results of ROC analysis: 2.44 * Isoleucine – Glycine; area under ROC-curve 0.8122 and 3.16 * Phenylalanine – Glycine, area under ROC-curve 0.8061. Plasma AA spectrum evaluation could be a new promising metabolic marker for AF paroxysm in CAD patients. Altered AA levels point to the depth of pathogenetic changes during AF paroxysm formation: disorders of AA metabolism with branched chain (isoleucine, leucine, valine), aromatic AA (phenylalanine, tyrosine), glutamine/glutamate, glycine/serine and glycine/methionine metabolism. A strong reliable connection between plasma AA spectrum and gut microbiota metabolites (trimethylamine, trimethylamine-N-oxide, total amount of fecal short chain fatty acids) was detected.*

Реферат. Амінокислотний спектр плазми крові як важлива метаболічна характеристика стану пацієнтів з ішемічною хворобою серця та фібриляцією передсердь. Мельничук І.О., Лизогуб В.Г. *Метою нашої роботи була оцінка особливостей амінокислотного (АК) спектра плазми в пацієнтів з ішемічною хворобою серця (ІХС) та фібриляцією передсердь (ФП) та перевірка їх зв'язку з відомими кардіометаболічними факторами ризику та метаболітами мікробіому кишківника. Триста пацієнтів були розподілені на три групи: перша – 149 пацієнтів з ІХС без аритмій, друга – 124 пацієнти з ІХС та пароксизмом ФП та контрольна група – 27 пацієнтів без ІХС та порушень серцевого ритму. Рівень АК у плазмі визначали методом іонообмінної рідинної колонкової хроматографії. Досліджено зміни АК спектра плазми в пацієнтів з ІХС та пароксизмом ФП: деякі АК (глутамат, глутамін, гліцин, аланін, валін, тирозин) та їх комбінації (ізолейцин+лейцин/валін, гліцин+серин, гліцин/метіонін, фенілаланін/тирозин, глутамін/глутамат) мали значні зміни в пацієнтів другої групи та мали кореляції з кардіометаболічними факторами ризику (гліцин, валін, аргінін, глутамат, ізолейцин, аланін, метіонін ($p<0,05$)). Були виявлені комбінації АК плазми, які можна використовувати як ранній маркер пароксизму ФП у пацієнтів з ІХС за результатами ROC-аналізу: 2,44 * ізолейцин – гліцин; площа під ROC-кривою 0,8122; 3,16 * фенілаланін – гліцин, площа під ROC-кривою 0,8061. Оцінка АК спектра плазми може бути новим перспективним метаболічним маркером розвитку пароксизму ФП у пацієнтів з ІХС. Змінені рівні АК вказують на глибину патогенетичних змін при формуванні пароксизму ФП: через порушення метаболізму АК з розгалуженим ланцюгом (ізолейцин, лейцин, валін), ароматичних АК (фенілаланін, тирозин), глутамін/глутамат, гліцин/серин та гліцин/метіонін співвідношень. Виявлений сильний міцний зв'язок між АК спектром плазми та метаболітами кишкової мікробіоти (триметиламіном, триметиламін-N-оксидом, загальною кількістю фекальних коротколанцюгових жирних кислот).*

Metabolomics studies focus on small molecule metabolites (sugars, nucleotides, amino acids, lipids) that impact the host metabolome while their biochemical functions have shown promise for studying host-gut microbiota interactions. Today new diagnostic methods give us a possibility to reveal specific metabolic signature of different diseases that enable to diagnose, predict disease course, and guide treatment strategies [1].

Gut microbiome regulates different functions in the host organism, including lipid and protein metabolism. Some studies have proved connection of gut dysbiosis with cardiovascular pathology, such as atherosclerosis, arterial hypertension, heart failure, platelet hyperactivity, vascular dysfunction [2]. Gut-heart axis can be altered and this leads to hyperactivation of sympathetic heart innervation through trimethylamine-N-oxide action (TMAO) [3]. Gut microbiota affects our health by its metabolites: TMAO and trimethylamine (TMA), short chain fatty acids (SCFA), bile acids, uremic toxins, lipopolysaccharide [4, 5]. Gut microbiota plays an important role in coronary artery disease (CAD) pathogenesis through several mechanisms: foam cell formation, inflammation, lipid metabolism, platelet hyperreactivity and thrombosis, endothelial dysfunction [7]. Several studies also classify plasma amino acids (AA) as gut microbiota metabolites, especially branched chain and aromatic acids [6, 7].

Atrial fibrillation (AF) is the most common arrhythmia in the world. CAD also is a widely spread cardiovascular pathology. Their combination is associated with increasing risk of morbidity and mortality. Both of them are characterized by the similar risk factors such as lipid profile, obesity, inflammatory diseases, heart failure, diabetes mellitus, arterial hypertension etc. All these factors are associated with special metabolic profile [4, 8]. Several studies analyzed plasma AA profile in AF patients, but their data are controversial [9, 10].

Plasma AA profile investigations are a new promising biomarker for the detection and screening AF paroxysm development in CAD patients [9, 10]. Its changes are associated with different cardiovascular and endocrine pathology, that are known AF and CAD risk factors [10, 11]. According to the previous studies CAD is associated with alterations in tyrosine, glycine, threonine and valine levels [9]. Also, changes in branched chain AA metabolism are commonly associated with CAD, aromatic AA changes are connected with diabetes mellitus [12], but recent data about concomitant AF are controversial, forcing us to further investigate of metabolic profile AF patients [9]. So, deeper pathophysiological investigations of AF paroxysm development are promising and of actual scientific interest.

The aim of our study was to estimate the peculiarities of plasma amino acid spectrum in coronary artery disease patients with atrial fibrillation and to check their connections with another gut microbiota metabolites – trimethylamine, trimethylamine-N-oxide, total amount of fecal short chain fatty acids, clinical and laboratory findings.

MATERIALS AND METHODS OF RESEARCH

300 patients were enrolled 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. CAD and AF diagnosis were based on latest European Sosiety of Cardiology guidelines [13, 14]. Inclusion criteria were based on results of objective clinical investigation (typical patients' compliance and history), resting 12 lead electrocardiogram (ECG), transthoracic echocardiography, ultrasound of the carotid arteries, lipid profile, invasive coronary angiography. To diagnose CAD, we selected patients in whom during invasive coronary intervention by anamnesis morbi, presence of atherosclerotic plaques was revealed. AF paroxysm was found by resting 12 lead ECG. ECG was done by CardioLab ECG complex (Kharkov, 2017). Carotid ultrasound and echocardiography were done by Toshiba Aplio 400 color Doppler ultrasound system (Japan, 2016). Exclusion criteria: reported malignancies, chronic kidney disease (glomerular filtration rate, GFR <60 mL/min), valvular AF, heart failure Class III to IV (by New York Heart Association), left ventricular dysfunction (ejection fraction <45%), thyroid pathology, inflammatory bowel disease, irritable bowel syndrome, vegetarians and vegans, pregnancy, probiotics and antibiotics taking for a month before the study. No significant difference in risk factors at baseline level were seen between investigated groups, they were comparable ($p>0.05$). Informed consent was obtained from all subjects in accordance with the Declaration of Helsinki. The study was conducted at the base of the Kyiv City Clinical Hospital No. 12 (protocol No. 8 from 22/08/2018) and was approved by the ethical commission. All investigated patients signed informed consent, the latter was approved at the same ethical commission meeting. Baseline characteristics of study sample are shown in Table 1.

By the data presented in Table 1, investigated groups are comparable by the mean clinical characteristics: age, body mass index (BMI), etc. Significant increase of uric acid levels (by 22.66% and 30.53% respectively) and decrease of glomerular filtration rate (by 26.16% and 19.38% respectively) was in the first and second groups as compared with control group (CG).

Table 1

Baseline characteristics of study sample, mean ± mean standard deviation

Characteristic /group	Group I	Group II	CG	p1-2	p2-CG	p1-CG
Age (years)	67.71±4.73	67.96±6.46	56.25±4.17	p>0.05	p>0.05	p>0.05
BMI (kg/m^2)	27.02±3.69	26.93±2.87	28.12±4.05	p>0.05	p>0.05	p>0.05
Uric acid (mmol/l)	380.5±95.38	404.9±142.7	310.2±90.79	p>0.05	p<0.05	p<0.05
Total bilirubin (mmol/l)	11.3±2.85	12.4±3.06	11.7±2.11	p>0.05	p>0.05	p>0.05
GFR (ml/min)	62.03±5.88	67.73±8.14	84.01±10.02	p>0.05	p<0.05	p<0.05

Also, lipid profile of investigated patients was compared. Data are shown in Table 2.

According to the obtained data, in the first and second group there was a significant increase of total cholesterol (TC) (32.64% and 43.06% respectively), triglycerides (TG) (80.36% and 55.36% respectively), low density lipoproteins (LDL) (70.78% and 72.73% respectively), lipoprotein α (Lpα) (41.17% and 54.95% respectively), apolipoprotein B (ApoB) (85.12% and 140.50% respectively) and decrease of high density lipoprotein (HDL) (16.09% and 29.31% respectively) levels compared with CG (p<0.05). In the second group significant increase of ApoB (29.91%) compared with first group was found.

Inflammatory markers and data of gut microbiota metabolites were checked. Results are shown in Table 3.

Table 2

Lipid profile of investigated groups, mean ± mean standard deviation

Characteristic/ group	Group I	Group II	CG	p1-2	p2-CG	p1-CG
TC (mmol/l)	5.73±1.44	6.18±1.29	4.32±0.47	p>0.05	p<0.05	p<0.05
TG (mmol/l)	2.02±0.87	1.74±0.63	1.12±0.60	p>0.05	p<0.05	p<0.05
LDL (mmol/l)	2.63±0.86	2.66±0.91	1.54±0.68	p>0.05	p<0.05	p<0.05
HDL (mmol/l)	1.46±0.49	1.23±0.56	1.74±0.63	p>0.05	p<0.05	p<0.05
Lpα (mg/dl)	22.53±5.73	24.73±7.70	15.96±2.74	p>0.05	p<0.05	p<0.05
Apo A1 (g/l)	2.02±0.51	2.34±0.88	1.62±0.62	p<0.05	p<0.05	p<0.05
Apo B (g/l)	2.24±0.64	2.91±0.79	1.21±0.45	p<0.05	p<0.05	p<0.05

Table 3

Inflammatory markers and gut microbiota metabolites of investigated groups, mean ± mean standard deviation

Characteristic/ group	Group I	Group II	CG	p1-2	p2-CG	p1-CG
CRP, mg/l	2.15±0.99	3.03±1.05	0.91±0.27	p<0.05	p<0.05	p<0.05
IL-6, pg/ml	2.66±0.81	3.27±0.87	1.61±0.20	p<0.05	p<0.05	p<0.05
TMA	21.89±2.64	25.42±1.90	17.87±1.97	p<0.01	p<0.01	p<0.01
TMAO	2.52±0.98	3.97±0.71	1.68±0.33	p<0.01	p<0.01	p<0.01
Total amount of fecal SCFA	2089.00±207.20	1732.00±114.42	2964.00±245.86	p<0.01	p<0.01	p<0.01

Significant increase of CRP (136.26% and 232.97% respectively), IL-6 (65.22% and 103.11% respectively), TMA (22.50% and 42.25% respectively), TMAO (50.00% and 136.31% respectively) levels and decrease of SCFA (29.52% and 41.57% respectively in the first and second groups was compared with CG ($p<0.05$). In the second group significant increase of CRP (40.93%), IL-6 (22.93%), TMA (16.13%), TMAO (57.54%) levels and decrease of total amount of SCFA (17.09%) were detected in comparison with a first group.

Plasma AA level was detected by method of ion exchange liquid column chromatography [15] – such AA were identified: lysine, histidine, arginine, ornithine, taurine, asparaginate, threonine, serine, glutamate, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, glutamine, ammonia. Blood sampling from patients was taken on an empty stomach from the cubital vein on the day of hospitalization. Results were presented as mean ± standard error for continuous variables or median [95% confidence interval (CI)] for categorical variables. Variables distribution for normality is checked by the Pearson criterion. If we got even one data distribution different from the normal between compared data, we used non-parametric analysis statistics methods (Kruskel-Wallis rank univariate analysis in case not normal distribution). Pearson's rank correlation coefficient was calculated. Analysis of ROC-curve for lipid and inflammatory indexes and linear regression model were done [16, 17]. All calculations were done in MATLAB R2014a (License number 271828).

RESULTS AND DISCUSSION

Plasma AA spectrum in CAD patients with and without AF was compared with CG (group of patients without CAD and AF). A general overview of plasma AA levels in the investigated groups is presented in Table 4.

Comparing medians of obtained results we got the following data ($p<0.05$):

1) In CAD group patients in comparison with CG, a reliable decrease of absolute glycine (83,5%), valine (18.10%) and alanine (74.86%) levels;

2) In comparable (CAD+AF) group patients in comparison with CG, a reliable increase of absolute glutamate (43.60%) level and depletion of absolute glycine (85.16%), valine (25.75%), alanine (72.73%), tyrosine (63.55%) and glutamine (68.47%);

3) In comparable group (CAD+AF) patients in comparison with CAD group, a reliable increase of absolute glutamate (35.14%) level and decriase of absolute glycine (10.07%) level.

Table 4

Plasma amino acid spectrum in CAD patients with or without AF compared with control group, mean [95% CI], mkmol/l

Characteristic/ group	CAD	CAD+AF	CG	p1-2	p2-3	p1-3
Lysine	25.35 [19.44; 133.33]	29.84 [19.36; 112.24]	91.83 [17.18; 181.32]	p>0.05	p>0.05	p>0.05
Histidine	11.52 [9.48; 53.04]	10.32 [9.60; 46.48]	46.49 [5.56; 63.38]	p>0.05	p>0.05	p>0.05
Arginine	13.13 [8.57; 87.5]	22.96 [9.12; 79.55]	62.09 [6.63; 119.32]	p>0.05	p>0.05	p>0.05
Ornithine	16.04 [10.01; 122.22]	22.09 [11.78; 88.00]	75.59 [7.85; 179.89]	p>0.05	p>0.05	p>0.05
Taurine	12.04 [4.8; 29.41]	7.87 [4.30; 45.29]	23.97 [3.28; 53.37]	p>0.05	p>0.05	p>0.05
Asparaginate	4.82 [1.42; 6.08]	3.95 [1.83; 4.39]	4.43 [0.79; 9.39]	p>0.05	p>0.05	p>0.05
Threonine	21.19 [12.87; 63.22]	17.65 [14.23; 69.54]	61.82 [10.97; 91.46]	p>0.05	p>0.05	p>0.05
Serine	15.08 [13.7; 52.17]	20.64 [13.49; 29.37]	60.18 [11.51; 103.45]	p>0.05	p>0.05	p>0.05
Glutamate	18.67 [15.64; 29.41]	25.23 [21.51; 36.76]	17.57 [7.34; 20.15]	p<0.05	p<0.05	p>0.05
Proline	25.00 [16.33; 63.46]	23.33 [16.67; 82.50]	80.23 [13.33; 115.38]	p>0.05	p>0.05	p>0.05
Glycine	31.18 [20.95; 147.90]	28.04 [21.50; 44.82]	189.00 [56.56; 281.40]	p<0.01	p<0.01	p<0.05
Alanine	57.86 [45.64; 145.29]	56.25 [48.31; 131.65]	206.28 [40.47; 345.24]	p>0.05	p<0.01	p<0.05
Cysteine	6.52 [5.32; 78.95]	5.69 [5.21; 39.47]	44.83 [5.32; 88.45]	p>0.05	p>0.05	p>0.05
Valine	28.56 [22.44; 142.86]	25.89 [19.48; 137.50]	34.87 [13.97; 82.86]	p>0.05	p<0.05	p<0.05
Methionine	2.71 [2.07; 9.18]	3.97 [2.64; 11.09]	6.03 [2.37; 16.13]	p>0.05	p>0.05	p>0.05
Isoleucine	8.33 [5.78; 31.5]	11.02 [6.86; 36.17]	31.38 [5.34; 46.88]	p>0.05	p>0.05	p>0.05
Leucine	16.35 [12.70; 61.54]	23.07 [12.90; 63.46]	51.87 [12.90; 92.31]	p>0.05	p>0.05	p>0.05
Tyrosine	8.01 [5.84; 35.71]	7.69 [5.96; 15.07]	21.10 [9.57; 44.12]	p>0.05	p<0.05	p>0.05
Phenylalanine	6.79 [6.08; 23.53]	12.14 [6.69; 24.12]	17.64 [5.56; 29.41]	p>0.05	p>0.05	p>0.05
Glutamine	78.22 [57.14; 337.26]	74.01 [51.19; 164.44]	234.79 [96.18; 398.53]	p>0.05	p<0.05	p>0.05
Ammonia	15.00 [11.22; 77.78]	27.97 [17.50; 66.95]	36.985 [9.06; 83.76]	p>0.05	p>0.05	p>0.05

For deeper understanding of pathogenetic ways of the amino AA exchange in patients with CAD and AF different plasma AA groups were investigated. Their meanings are presented in Table 5.

According to the published data branched chain amino acids (BCAA) metabolism plays an important role in development of diabetes mellitus and dyslipidemia [9, 13], also it is closely connected with gut microbiota condition [18]. So, isoleucine+leucine/valine ratio was checked in investigated groups, this index was significantly lower in CAD and CAD+AF patients than in CG ($p<0.05$). Also, the role of glycine, serine and methionine in lipid exchange, gut microbiota condition is still interesting [9, 12, 19]. Glycine+serine volume was significantly lower in CAD and CAD+AF patients than in CG ($p<0.05$). Also, glycine/methionine ratio was significantly lower in CAD and CAD+AF patients than CG and in CAD+AF patients compared with CAD ($p<0.05$). Aromatic amino acids (AAA) are closely associated with lipid exchange alterations [20] and atherosclerosis [21]. Phenylalanine/tyrosine ratio significantly increased in CAD and CAD+AF patients compared with CG and in CAD+AF patients compared with CAD ($p<0.05$). Glutamine/glutamate ratio was closely connected with BCAA metabolism [18] and it was increased in CAD+AF patients compared with CAD and CG ($p<0.05$).

Table 5

Plasma AA spectrum combinations in CAD patients with or without AF compared with control group, mean [95% CI], mkmol/l

Characteristic /group	Group I	Group II	CG	p1-2	p2-CG	p1-CG
Isoleucine+Leucine/Valine	0.90 [0.70; 0.96]	0.85 [0.78; 0.98]	1.57 [1.11; 2.86]	p>0.05	p<0.05	p<0.05
Glycine+Serine	47.40 [34.78; 188.10]	56.55 [39.73; 95.82]	208.52 [28.07; 363.95]	p>0.05	p<0.05	p<0.05
Glycine/Methionine	11.11 [8.37; 16.59]	6.20 [4.91; 10.16]	13.78 [10.69; 25.67]	p<0.05	p<0.05	p<0.05
Phenylalanine/Tyrosine	0.91 [0.72; 1.16]	1.15 [0.98; 1.40]	0.69 [0.45; 1.04]	p<0.05	p<0.05	p<0.05
Glutamine/Glutamate	5.08 [3.90; 8.18]	6.99 [6.17; 9.08]	3.59 [2.13; 7.80]	p<0.05	p<0.05	p>0.05

To our opinion, significant changes of plasma AA spectrum levels should be a promising prognostic marker of AF paroxysm in CAD patients. ROC-analysis was performed individually for each plasma AA and their combinations for further validation of their diagnostic values. We found that optimal plasma AA combinations can be used as prognostic markers of risk of AF paroxysm in CAD patients. The area under the ROC curve exceeded 0.75 for different AA and their combinations, while the p-value was significant ($p<0.05$). The ROC-analysis validated the prognostic value of plasma levels of AA in AF and CAD. Results are presented in Figure 1.

2.44 * isoleucine – glycine and 3.16 * phenylalanine – glycine shows an excellent evaluation opportunity for AF paroxysm diagnostics and prediction in CAD patients. Moreover, it showed the importance of glycine, alanine, BCAA (isoleucine, valine) and AAA (phenylalanine) in AF paroxysm development in CAD patients, that further validated the diagnostic value of listed AA.

The correlation analysis between plasma AA spectrum and cardiometabolic risk factors of the examined groups was done. Spearman's correlation analysis was used to explore their correlations with species number. All correlations are presented in the Figure 2.

The largest number of correlations was revealed between plasma AA spectrum and such clinical characteristics as total amount of fecal SCFA (total number=12), ApoB (total number=10), CRP (total number=9), TMAO (total number=8) and TMA (total number=8) levels. The highest number of correlations was between glycine (total number=9), valine (total amount=8), arginine (total number=7), glutamate (total number=7), isoleucine (total number=7), alanine (total number=6), methionine (total number=6) and clinical-laboratory changes.

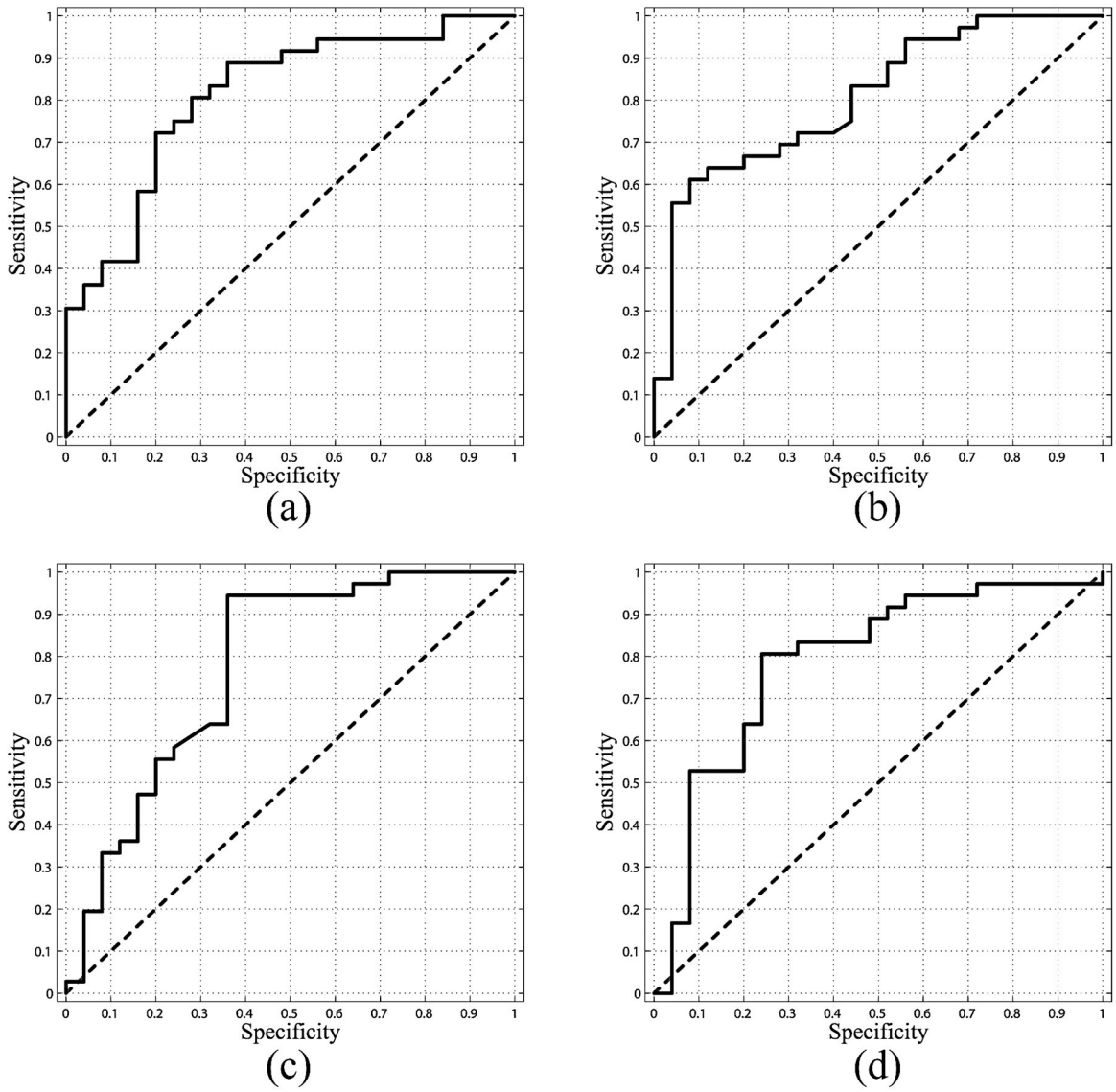

Fig. 1. ROC-curve analysis for respective AA and their combinations in CAD and CAD+AF groups p<0.05:
A – 2.44 * isoleucine – glycine, area under ROC-curve 0.8122;
B – 3.16 * phenylalanine – glycine, area under ROC-curve 0.8061;
C – 106 * valine/glycine – alanine, area under ROC-curve 0.7750;
D – glycine – 19.4 * alanine/isoleucine, area under ROC-curve 0.7889

This study evaluates spectrum of plasma AA as an important part of metabolic profile and their network in pathogenesis of AF paroxysm in CAD patients. Their role was checked by the following observations: several plasma AA (glutamate, glutamine, glycine, alanine, valine, tyrosine) and their combinations (isoleucine+leucine/valine, glycine+serine, glycine/methionine, phenylalanine/tyrosine, glutamine/glutamate); changes in CAD+AF patients; their strong correlations with mean clinical-laboratory characteristics of groups (glycine, valine, arginine, glutamate, isoleucine, alanine, methionine); confirmed role of some AA combinations as a marker of AF paroxysm in CAD patients (2.44 * isoleucine – glycine; area under ROC-curve 0.8122 and 3.16 * phenylalanine – glycine; area under ROC-curve 0.8061).

Plasma AA spectrum is an important marker of human metabolism. Its changes are common for different pathological conditions including AF [9, 10] and atherosclerosis [11, 12]. But obtained data are still controversial in different studies, and the importance of further investigations is undoubted. Moreover, in our work we detected the optimal AA combinations that can help us to predict AF paroxysm in CAD patients, that was not done before.

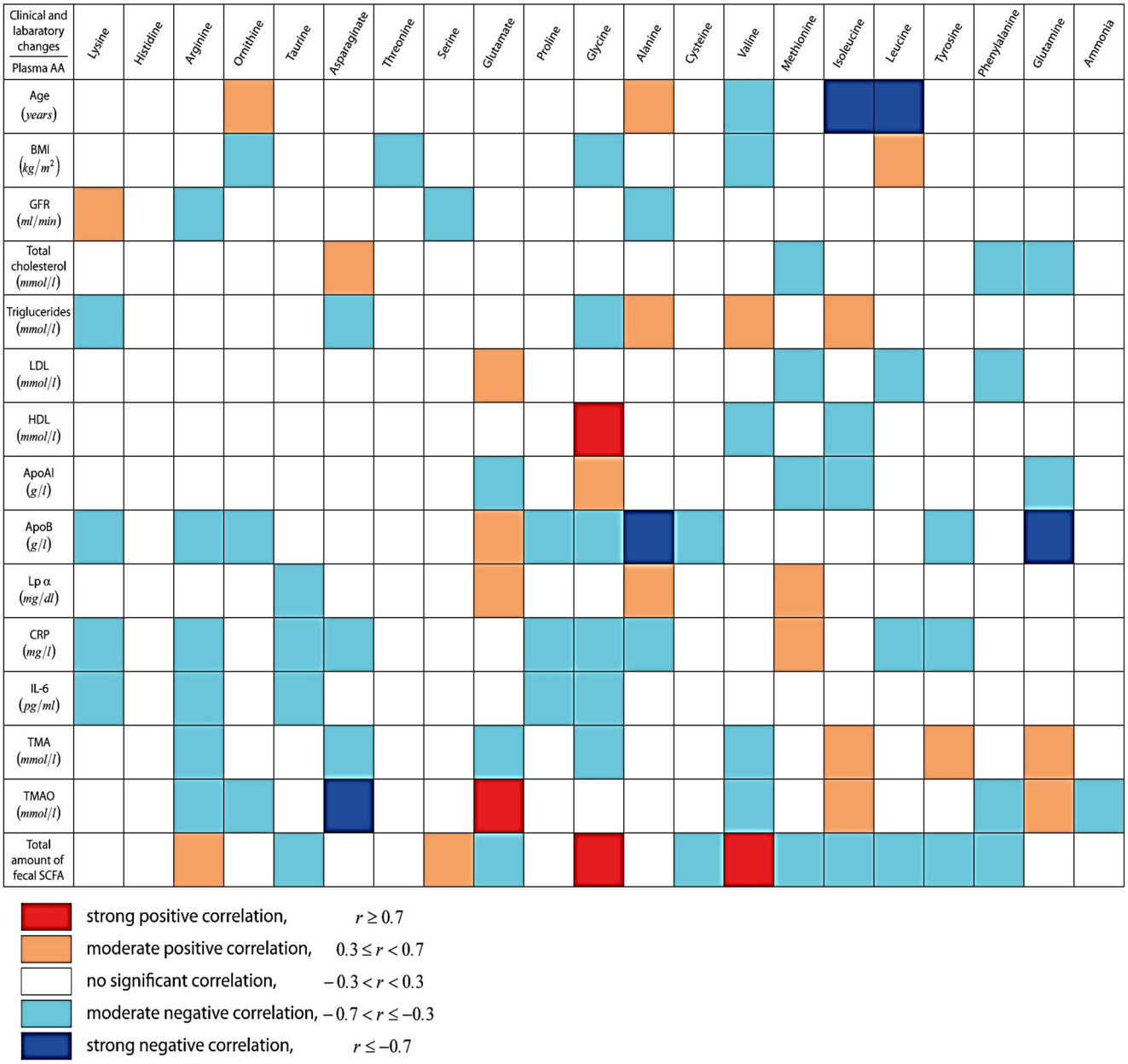

Fig. 2. Matrices of heatmap correlation between plasma AA levels and cardiometabolic risk factors, p<0.05

BCAA (isoleucine, valine, leucine) and AAA (phenylalanine, tyrosine) metabolism is closely linked with gut microbiota condition [22], in our study they correlated with such gut microbiota metabolites as TMA, TMAO and total amount of fecal SCFA.

In several animal studies BCAA catabolism is obviously impaired in CAD cases. BCAA are accumulated in left ventricular myocardium, this leads to its hypertrophy by activation of myocardial mTOR signal. In myocardium, BCAA acts as an important nutrient signal to promote protein synthesis, cells growth and metabolism. Increase of myocardial BCAA is directly linked with heart failure development and left ventricular remodeling. At the same time, in patients with MI plasma BCAA level commonly decreased [23]. Moreover, valine is crucial substrate in platelets activation by catabolic way of valine/α-ketoisovaleric acid [24]. It is widely known that platelets activation is common for CAD and AF [2, 13, 14]. On the other hand, in animal studies dietary valine improved gut microbiota composition (increased number of the *Akkermansia* and *Bifidobacterium)* and decreased of *β*-oxidation of fatty acids [25].

Host-microbiome interaction is closely connected with AAA metabolism. AAA take part in neurotransmitters (dopamine, norepinephrine, adrenaline, melatonin) and hormones (thyroxine) synthesis. Indole-3-aldehide and indole propionic acid, which are phenylalanine metabolites, produced by *Bacteroides* and *Rumminococcus*, provide intestinal barrier integrity. They can decrease inflammation, insulin sensitivity and salt sensitive hypertension. Tyramine, which is tyrosine metabolite, produced by *Rumminococcus*, has vasodilatating and neuromodulating effects. On the other hand, tyrosine is precursor of 4-hydroxyphenilacetic acid, produced by *Faecalibacterium* and *Bacteroides*, and is genotoxic for human enterocytes [26].

Glutamine is the most abundant AA in intestinal endothelium. It improves intestinal mucosal integrity, stimulates immune response by increasing proportion of CD8+ and CD3+ in lymphatic nodes, modulates nucleotides biosynthesis and energy metabolism. Glutamine additive improves gut microbiota composition: increases ration of *Firmicutes* to *Bacteroides* and decreases *Actinobacteria* level. It can initiate AA metabolism in small intestine by bacterial species [27]. According to some data, glutamine decline is associated with AF due to increase of heat shock proteins level [28].

In our study alterations of glycine metabolism play an important role in AF paroxysm pathogenesis. According to published data, about 30% of glycine supplied with food is metabolized by the microbiota of the small intestine, and by both gram-positive and gram-negative flora. However, it is still unknown what determines the activity of glycine degradation by intestinal microbiota. For example, the activity of glycine degradation increases in patients with type 2 diabetes. It was found that patients who took probiotics (bifido and lactobacilli) had a lower concentration of glycine in their feces. An increase in the content of formic acid in the feces is a sign of increased glycine degradation under the influence of the gut microbiome. On the other hand, the state of the intestinal microbiome can influence the metabolism of glycine in the host's body due to changes in the expression of enzymes. It was estimated that intestinal dysbiosis has an effect on the development of NASH by reducing the content of circulating glycine. Also, obesity and metabolic syndrome are closely associated with a decrease in circulating glycine and impairment of the gut microbiome [29].

CONCLUSIONS

1. Plasma amino acids spectrum evaluation can be a new promising metabolic marker/pattern of atrial fibrillation paroxysm in coronary artery disease patients.
2. Altered amino acids levels indicate the depth of pathogenetic changes during atrial fibrillation paroxysm formations as it is characterized by disorders in branched-chain amino acids (isoleucine, leucine, valine), aromatic amino acids (phenylalanine, tyrosine), glutamine/glutamate, glycine/serine and glycine/methionine metabolism.
3. A strong reliable connection between plasma amino acids spectrum and known gut microbiota metabolites (trimethylamine, trimethylamine-N-oxide, total amount of fecal short chain fatty acids) was detected.
4. Highly validated plasma amino acids combinations that can be used as an early marker for atrial fibrillation paroxysm in coronary artery disease patients ($p<0,05$) were proposed in this study: 2.44 * isoleucine – glycine; area under ROC-curve 0.8122 and 3.16 * phenylalanine – glycine, area under ROC-curve 0.8061.

Contributors:

Melnychuk I.O. – data curation, formal analysis, writing – review & editing, project administration;

Lyzogub V.H. – conceptualization, methodology.

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Conflict of interests. The authors declare no conflict of interest.

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