Targeted metabolomic profiling for acute myocardial infarction pathogenesis

Sergey S. Markin
phospholipovit@ibmc.msk.ru

Institute of Biomedical Chemistry

Elena A. Ponomarenko
Institute of Biomedical Chemistry

Yulia A. Romashova
Institute of Biomedical Chemistry

Tatiana O. Pleshakova
Institute of Biomedical Chemistry

Sergei V. Ivanov
Institute of Biomedical Chemistry

Valery V. Beregovykh
Institute of Biomedical Chemistry

Sergey L. Konstantinov
Belgorod Regional Clinical Hospital of St. Joseph

Galina I. Stryabkova
Belgorod Regional Clinical Hospital of St. Joseph

Zhanna Yu. Chefranova
Belgorod Regional Clinical Hospital of St. Joseph

Yuri A. Lykov
Belgorod State National Research University

Irina M. Karamova
Ufa Emergency City Clinical Hospital

Anton G. Koledinskii
Peoples' Friendship University of Russia

Ksenia M. Shestakova
Laboratory of Pharmacokinetics and Metabolomic Analysis, Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University)

Pavel A. Markin
Laboratory of Pharmacokinetics and Metabolomic Analysis, Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University)

Natalia E. Moskaleva
Laboratory of Pharmacokinetics and Metabolomic Analysis, Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University)

Svetlana A. Appolonova

Laboratory of Pharmacokinetics and Metabolomic Analysis, Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University)

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Abstract

Background. Acute myocardial infarction (AMI) represents a major cause of morbidity and mortality worldwide. Metabolomics investigation may be useful in the AMI pathogenesis research.

Materials and methods. The article describes a comprehensive study of the AMI through the targeted metabolomic profiling. A total of 195 subjects were enrolled in the study, consisting of 68 AMI patients, 84 IHD patients and 43 non-CVD subjects. Metabolomic profiling was conducted, involving the quantitative analysis of 87 endogenous metabolites in plasma.

Results. We identified 36 significantly changed metabolites in AMI, which included increased cystathionine and dimethylglycine and the decreased asymmetric dimethylarginine (ADMA) and arginine. It was found, that patients with AMI had significantly lower concentration of short chain acylcarnitines as compared to IHD and non-CVD patient. In patients with AMI concentration of xanthurenic acid and 3-OH-kynurenine was significantly decreased, as compared to IHD patients and non-CVD subjects. Norepinephrine was significantly decreased in patients with AMI and IHD, whereas its end-product – vanillylmandelic acid (VMA) – significantly increased. Based on the differences in the constructed weighted correlation networks, there were found new significant ratios of the metabolites. Among 23 established significantly altered metabolite ratios 14 ratios between non-CVD vs AMI and 17 ratios between IHD vs AMI were found. 9 ratios between non-CVD vs AMI and IHD vs AMI and 2 ratios between non-CVD vs IHD vs AMI were coincided.

Conclusion. Obtained findings may pave the way for new insight of AMI pathogenesis and ultimately improving clinical outcomes.

1. Introduction

Acute myocardial infarction (AMI) is the most common manifestation of ischemic heart disease (IHD) and represents a major cause of morbidity and mortality worldwide. AMI is a state of acute critical condition, when the coronary artery and its main branches are occluded and are characterized by severe stenosis, causing atherothrombosis and significant decrease and interruption of blood flow. AMI refers to necrosis of the myocardium caused by severe ischemia. Myocardial ischemia provides strong impact on the entire thickness of the ventricular muscle, known as transmural injury, or may affect only the inner layer of the ventricle (subendocardial ischemia or infarction). This process results in adverse ventricular remodeling. The viable myocardium undergoes hypertrophic growth, as a compensatory measure, aiming to improve myocardial contractility. Impaired pumping function leads to the development of heart failure [1].

Clinical diagnosis of AMI is mainly based on clinical symptoms of the patient, electrocardiogram, coronary angiography, nuclear magnetic resonance with gadolinium contrasting, myocardial necrosis markers, and other auxiliary examinations. These diagnostic strategies are effective, but don't cover the insight of AMI due to the fact, that many patients had different mass of necrosis and late gadolinium enhancement shows grey zone, consisting of ischemic and alive myocardium in the early stage of AMI, and the electrocardiogram may not be specific for these changes. Clinical biomarkers used for AMI diagnostic, such as troponin and creatine kinase, require detection several hours after the onset of the disease and required only the zone of necrosis [2]. Therefore, there is a need to develop biomarkers that can offer timely, non-invasive, and reliable prediction of the AMI occurrence, thus improving clinical diagnosis and prognosis of AMI, using metabolomics investigation studies [3].

Metabolites research can reflect the physiological or pathological state of the organism. The targeted metabolomic profiling, utilized in the presented study, is based on the absolute quantification of various metabolites related to different chemically and biochemically related classes of endogenous metabolites. This method provides high levels of sensitivity and selectivity in comparison to nontargeted metabolomic analysis.

The presented study is aimed to identify significant biochemical changes in the AMI pathogenesis in comparison with IHD patients and subjects without any cardiovascular pathologies in order to underline potential biomarkers, as well, as to identify new insights in the AMI pathogenesis.

2. Material and methods

2.1 Study design

Inclusion and exclusion criteria of the study are presented in the Table 1.

Table 1
Inclusion and exclusion criteria

Inclusion Criteria:	**• Men and women aged 18 years and older;** **• ST-elevation myocardial infarction (STEMI), angina pectoris functional class III according to Canadian Cardiovascular Society classification;** **• Availability of signed and dated informed consent of the patient to participate in the study.**
Exclusion Criteria:	• Myocardial infarction without ST-elevation; • Angina pectoris functional class I, II or IV; • Type 1 Diabetes mellitus; • Acetaminophen, all vitamins, minerals, amino acids, dietary supplements, including sports drinks and energy drinks, creatinine, alpha-ketoglutarate, malic acid, citric acid, maleic acid, orotic acid consumption during 4 days before blood sampling. Sweeteners (aspartame, among others), monosodium glutamate and alcohol intake 24 h before blood sampling (Non-CVD group and IHD group).* • Any other diseases or conditions that, in the opinion of the investigator, may distort the results of the study and limit the patient's participation in the study.
*Patients with AMI, who violated their diet or consumed alcohol, were excluded from data analysis after blood sampling	

2.2 Ethical considerations

All conducted experiments were approved by the Ethics Committee of Belgorod Regional Clinical Hospital of St. Joseph, Belgorod, Russia (protocol No.10 from 16 of November, 2015) in conformity with the ethical principles for medical research involving humans stated in the Declaration of Helsinki. Written informed consent was signed by all the participants before the beginning of the study.

2.3 Baseline characteristics of the participants

Baseline characteristics of the participants included measurements of weight, height, calculation body mass index (BMI), measurements of heart rate and blood pressure, determination of myocardial infarction localization.

2.4 Biochemical analysis

Fasted whole blood samples were received in the morning into ethylenediaminetetraacetic acid (EDTA) tubes, centrifuged during 20 min (2000 rpm, 4°C) to receive plasma and stored at -80°C. The biochemical analysis of the samples consisted of the analysis presented in [7] including measurements of total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, glucose, creatinine, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), troponin I, potassium, sodium, calcium, international normalized ratio (INR), activated partial thromboplastin time (APTT) and fibrinogen. Extra plasma aliquots were utilized for the metabolic analysis in the Laboratory of pharmacokinetics and metabolome analysis.

2.5 Chemicals and reagents

All solvents, standard solutions for metabolomic profiling (including isotopically-labeled) corresponded to those utilized in [7]. Briefly, standard solutions for metabolomic profiling, methanol, formic acid, bovine serum albumin (BSA) were received from Sigma-Aldrich (USA). Acetonitrile was purchased from Chromasolv® (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). Ultrapure water was received through the Millipore Milli-Q purification system (Millipore Corporation, Billerica, USA). Isotopically-labeled standard solutions for metabolic profiling Amino Acids and Acylcarnitines were received from MassChrom Non Derivatized 57000 Kit (Chromsystems, Germany), whereas isotope-labeled standard solutions for tryptophan catabolites profiling were from Toronto Research Chemicals (Canada).

2.6 Metabolomic profiling

Targeted metabolomic profiling of the samples was performed in accordance to presented method [3] and included quantitative analysis of 87 endogenous metabolites in patient's plasma (listed in the supplementary material, Table S1). Briefly, sample preparation of amino acids, intermediates of Arginine and Methionine metabolism consisted of protein precipitation with following instrumental analysis on Waters TQ-S-micro triple quadruple mass spectrometer (Waters Corp, Milford, CT, USA). Preparation of samples for acylcarnitine and tryptophan catabolite profiling consisted of liquid-liquid extraction followed by LC-MS/MS analysis. The applied methods were validated in accordance with the guidelines for bioanalytical method validation and included assessment of selectivity, linearity, precision and accuracy, recovery, matrix effect, and stability of the methods.

2.7 Echocardiography

Left ventricular ejection fraction (LVEF) was assessed by quantitative biplane Simpsons measurements.

2.8 Statistical analysis

All statistical analyses for characterization of biochemical and metabolic profiling measurements were performed using Python Stats package in accordance with the method presented in [7]. Briefly, variable distribution was assessed using the Shapiro-Wilk test. According to the variable distribution, the analysis of variance was performed using parametric student t-test and ANOVA test or using non-parametric Kruskal-Wallis test and Mann-Whitney U test. After Bonferroni adjustment for multiple comparisons the p-value, less than 0.05, was considered as significant.

2.9 Correlation network analysis

The weighted correlation network analysis was performed using debiased Sparse Partial Correlation algorithm (DSPC) by means of Cytoscape software [5] with Metscape plugin [6]. It calculated the relation between changes in metabolite levels as well as with clinical and anthropometric factors. We used previously approved method for age-adjustment correction of metabolic data in patients which were non-balanced on-age [7].

3. Results

3.1 Baseline characteristics of the participants

290 patients were screened for the study. 95 patients did not meet inclusion criteria and were ineligible (Fig. 1).

4 subjects from non-CVD group, 5 patients from IHD group and 3 patients from AMI group were excluded from the study due to diet violation (energy drinks consumption). 3 subjects from non-CVD, 3 patients from IHD group and 4 patients from AMI group were excluded due to alcohol consumption before blood analysis.

Patients with AMI had evidence of ST-segment elevation on their qualifying electrocardiogram (at least 2 mm in two contiguous peripheral or precordial leads, the average ST elevation was 3,58 ± 1,96 mm). AMI patients were treated with intravenous thrombolysis with alteplase or non-immunogenic staphylokinase, following by percutaneous coronary intervention (PCI) with stenting, as well, as with intravenous heparin (70 IE/kg), clopidogrel, acetylsalicylic acid and, if necessary, due to chest pain, either intravenous fentanyl, morphine or nitroglycerin. IHD patients used organic nitrates, β-blockers, calcium channel blockers, ACE inhibitors and statins.

Patients were considered as having IHD in case of angina pectoris functional class III in accordance with Canadian Cardiovascular Society classification and a combined dyslipidemia indicated by the increased levels of triglycerides and decreased levels of HDL-cholesterol [4]. IHD patients were treated with organic nitrates, β-blockers, calcium channel blockers, ACE inhibitors, ARBs and statins.

The non-CVD group included individuals having no risk factors of IHD or cardiovascular pathology.

All information regarding demographics, medical history, biochemical analysis and patient's treatment was kindly provided from the hospital internal database.

Among the considered patients, those having AMI and IHD, were older than subjects from the non-CVD group, as well, as were characterized by higher BMI values.

The troponin I level was significantly increased in AMI group vs the non-CVD and IHD group. The lipid analysis showed that total cholesterol was in normal range in all groups, but high triglycerides and low HDL-cholesterol were observed in AMI and IHD patients, that are characteristics of combined dyslipidemia.

The creatinine level was increased in IHD group vs the non-CVD and AMI group, nevertheless, it was in normal range. The measurements of ALT, AST and glucose were increased in AMI group. The coagulogram showed the normal range of INR, APTT and fibrinogen in all groups.

LVEF was decreased in patients with AMI and IHD as compared with non-CVD subjects.

More information, concerning the characteristics of patients, is represented in Table 2.

Table 2
Baseline characteristics of the participants

Variable	Non-CVD group (n = 36)	IHD group (n = 76)	AMI group (n = 61)	p-value		
				Non-CVD vs IHD	Non-CVD vs AMI	IHD vs AMI
Gender, m/f (%)	29/7 (80/20)	65/11 (86/14)	50/11 (82/18)			
Age, years	34 [26–44]	64 [58–71]	58 [54–66]	< 0.0001	< 0.05	0.10
Height, m	1.67 [1.62–1.72]	1.69 [1.64–1.75]	1.72 [1.68–1.76]	0.21	0.11	0.72
Weight, kg	69.0 [59.0–81.0]	84.4 [70.0–89.0]	85.3 [72.0–90.0]	< 0.0001	< 0.001	0.57
BMI, kg/m^2	24.6 [20.7–27.4]	29.5 [25.6-31.63]	28.8 [24.9–31.2]	< 0.0001	< 0.01	0.65
Heart rate/min	62.2 ± 10.8	65.8 ± 9.2	75.9 ± 14.7	0.51	< 0.05	< 0.05
Systolic BP, mm Hg	119.5 ± 5.6	120.3 ± 6.9	118.6 ± 8.2	0.82	0.59	0.62
Diastolic BP, mm Hg	75.9 ± 6.2	74.7 ± 5.9	74.7 ± 7.2	0.78	0.65	0.95
AMI localization -Anterior wall infarction -Inferior wall infarction -other	n/a	n/a	26 (42.7) 34 (55.7) 1 (1.6)	n/a	n/a	n/a
Troponin I, ng/ml	0.001 [0.00-0.005]	0.05 [0.02–0.07]	0.29 [0.08–1.03]	< 0.05	< 0.001	< 0.05
Total cholesterol, mmol/l	5.27 [4.81–5.93]	5.55 [4.50–6.20]	5.51 [4.62–6.21]	0.48	0.47	0.89
Triglycerides, mmol/l	1.10 [0.76–1.28]	1.73 [0.96–2.18]	1.82 [1.01–2.21]	< 0.01	< 0.01	0.51

Variable	Non-CVD group (n = 36)	IHD group (n = 76)	AMI group (n = 61)	p-value		
				Non-CVD vs IHD	Non-CVD vs AMI	IHD vs AMI
LDL-cholesterol, mmol/l	3.32 [2.71-4.00]	3.30 [2.92–4.10]	3.52 [3.00-4.22]	0.61	0.74	0.88
VLDL-cholesterol, mmol/l	0.51 [0.30–0.69]	1.00 [0.72–1.20]	0.97 [0.61–1.21]	0.01	0.01	0.95
HDL-cholesterol, mmol/l	1.51 [1.22–1.78]	1.08 [0.89–1.19]	1.22 [1.01–1.42]	< 0.001	< 0.01	< 0.05
Glucose, mmol/l	4.88 [4.59–5.30]	5.65 [4.70–5.88]	10.1* [7.4–10.9]	< 0.01	< 0.001	< 0.001
Creatinine, μmol/l	89.1 [79.4–97.2]	100.5 [85.5-111.6]	75.5 [62.5–85.6]	0.01	0.01	< 0.001
Urea, mmol/l	6.1 [6.1–6.3]	6.5 [5.3–7.4]	6.1 [5.0-7.1]	0.85	0.66	0.23
ALT, u/l	23.0 [20.0–24.0]	29.0 [20.0-34.5]	51.0 [24.3–60.7]	0.31	< 0.01	< 0.01
AST, u/l	25.0 [23.0–27.0]	36.0 [20.0–36.0]	159.1 [32–232]	0.66	< 0.01	< 0.01
CPK, u/L	10.2 ± 2.8	50.3 ± 12.1	328.4 ± 505.6	< 0.05	< 0.001	< 0.001
CPK-MV, u/L	3.1 ± 1.2	10.8 ± 3.7	39.8 ± 59.9	< 0.05	< 0.001	< 0.001
Potassium, mmol/l	4.63 [4.30–4.90]	4.41 [4.10–4.65]	3.72 [3.35–3.96]	< 0.05	< 0.001	< 0.001
Sodium, mmol/l	142.8 [141–145]	140.0 [138.0-142.2]	142.1 [141.0-143.7]	0.12	0.76	0.08
Calcium, mmol/l	-	2.18 [2.10–2.23]	0.91 [0.81–1.01]	-	-	< 0.01

Variable	Non-CVD group (n = 36)	IHD group (n = 76)	AMI group (n = 61)	p-value		
				Non-CVD vs IHD	Non-CVD vs AMI	IHD vs AMI
INR	1.09 [1.05–1.14]	1.23 [1.11–1.28]	1.13 [1.01–1.23]	< 0.05	0.53	0.05
APTT, s	27.2 [25.5–28.6]	31.5 [27.0-34.8]	42.0 [28.9–74.0]	0.19	< 0.05	< 0.05
Fibrinogen, g/L	2.25 [2.05–2.30]	3.28 [2.53–3.74]	3.40 [3.10–4.10]	< 0.05	< 0.01	0.21
LVEF, %	59.8 [60.2–64.5]	53.2 [45.0–60.0]	49.2 [39.0-52.3]	0.08	< 0.01	0.05

Baseline characteristics of the participants including n (%) or median and interquartile range [Q1; Q3] in the considered groups and corresponding p-values, characterizing statistically significant differences between groups

*Hyperglycemia is due to non-fasting blood samples was taken before angiography in AMI patients.

3.2 Correlation between levels of plasma metabolites and results of the biochemical analysis

Based on the conducted correlation analysis, we have identified, that short chain acyl carnitines had direct correlation with weight, glucose and AST, while potassium, calcium, magnesium and prothrombin showed inverse correlation. At the same time, medium-chain acylcarnitines were directly correlated with age, creatinine, urea, calcium, INR and were inversely correlated to LDL-cholesterol, glucose, ALT, AST, CPK and prothrombin.

Long-chain acylcarnitines had strong correlation with weight, BMI and INR. Long-chain carnitines were inversely correlated to prothrombin, calcium, potassium, glucose, and HDL-cholesterol levels. Creatinine had strong correlation with Choline, Citrulline, Cystathionine, SDMA, 3-Aminoisobutyric acid, 3-Hydroxyanthranilic acid, 3-Hydroxykynurenine, GABA, 5-Hydroxytryptophan, Acetylcholine, Anthanilic acid, Aspartic acid, Biopterin, 5-Hydroxyindoleacetic acid, Indole-3-acetic acid, Indole-3-acrylic acid, Indole-3-butyric acid, Indole-3-carboxaldehyde, Indole-3-lactic acid, Indole-3-propionic acid, Kynurenic acid, Kynurenine, Melatonin, Metanephrine, Neopterin, Norepinephirne, Normetanephrine, Quinolinic acid, Serotonin, Tryptophan, Tryptophol, Vanillylmandelic acid, Xanthurenic acid and Kyn/Trp.

HDL-cholesterol were strongly correlated with the level of several amino acids (Alanine, Arginine, Isoleucine, Methionine, Threonine), whereas sodium was inversely correlated with leucine, phenylalanine, serine, valine. 5HIAA, kynurenine, kynurenic acid, 3-Aminoisobutyric acid, kyn/trp had strong correlation with BMI and urea.

The correlation heat maps visualization of the relationships between the observed concentrations of the metabolites and corresponding biochemical analysis are presented in the Fig. 2 (A-D).

3.3 Differences in metabolic profiles of AMI, IHD patients and non-CVD subjects

First of all, for general overview of the received data, as well, as for outlier exclusion, we performed a principal component analysis (PCA) (Fig. 3). It has revealed, that groups may be partly separated from each other.

To identify metabolites, that significantly distinguished among the non-CVD, IHD and AMI patients, we performed using parametric and non-parametric comparison tests. Table 3 represents information regardless the significantly changed metabolites, containing information on class of the metabolite, direction of change and adjusted p-value.

Table 3
Meaningful metabolites selected based on the multiple hypothesis comparison with Bonferroni correction

Metabolite	Adj. p-value (among 3 groups)	Non-CVD vs IHD		Non-CVD vs AMI		IHD vs AMI	
		Direction (for IHD)	Adj. p-value	Direction (for AMI)	Adj. p-value	Direction (for AMI)	Adj. p-value
3-Amino isobutyric acid	< 0.01	increased	< 0.05		-		< 0.01
Choline	< 0.0001	increased	< 0.0001		-		-
Cystathionine	< 0.001	increased	< 0.001		-		-
Acetylcholine	< 0.01	increased	< 0.01		< 0.05		-
Dimethylglycine (DMG)	< 0.0001	increased	< 0.0001		< 0.0001		-
Methionine sulfoxide	< 0.0001		-	increased	< 0.0001	increased	< 0.0001
Vanillylmandelic acid	< 0.001		-	increased	< 0.01	increased	< 0.05
Norepinephirne			-		-	decreased	< 0.05
Anthranilic acid	< 0.0001	increased	< 0.001	increased	< 0.0001	increased	< 0.01
Serotonin	< 0.05		-	increased	< 0.001		-
Kynurenic acid	< 0.0001	increased	< 0.001		-		-
Kynurenine	< 0.01	increased	< 0.01		-		-
Tryptophol	< 0.0001		-	increased	< 0.0001	increased	< 0.0001
Xanthurenic acid	< 0.01		-		-	decreased	< 0.01
3-Hydroxykynurenine	< 0.001		-		-	decreased	< 0.001
Indole-3-propionic acid	< 0.01		-	decreased	< 0.01		
Symmetric dimethylarginine (SDMA)	< 0.01	increased	< 0.01	increased	< 0.05		
Biopterin	< 0.001		-	increased	< 0.01	increased	< 0.01
Citrulline	< 0.01		-		-	decreased	< 0.01
Glutamine	< 0.0001	increased	< 0.01	increased	< 0.0001	increased	< 0.05
Phenylalanine	< 0.001	increased	< 0.0001	increased	< 0.05		
Isoleucine7	< 0.05	increased	< 0.05		-		

Metabolite	Adj. p-value (among 3 groups)	Non-CVD vs IHD		Non-CVD vs AMI		IHD vs AMI	
		Direction (for IHD)	Adj. p-value	Direction (for AMI)	Adj. p-value	Direction (for AMI)	Adj. p-value
Leucine	< 0.01	increased	< 0.01		-	decreased	< 0.05
Valine	< 0.01	increased	< 0.01		-	decreased	< 0.05
N-methylmalonamic acid (NMMA)	< 0.0001	increased	< 0.05		-	decreased	< 0.001
Proline			-		-	increased	< 0.05
Asparagine	< 0.01		-		-	increased	< 0.01
Aspartic acid	< 0.0001		-	decreased	< 0.001	decreased	< 0.001
Methionine	< 0.001		-		-	decreased	< 0.001
Glycine	< 0.01	decreased	-	decreased	< 0.05	decreased	< 0.05
Carnitine (C0)	< 0.01	increased	< 0.01	increased	< 0.05		-
Adipoylcarnitine (C6-DC)	< 0.01	increased	< 0.01		-		-
Hydroxytetradecanoylcarnitine (C14-OH)	< 0.0001	increased	< 0.0001		-		-
Isovalerylcarnitine (C5)	< 0.0001	increased	< 0.01	increased	< 0.0001	increased	< 0.001
Palmitoylcarnitine (C16)	< 0.01		-	increased	< 0.01		-
Palmitoleyl carnitine (C16-1)			-	increased	< 0.05		-
Oleoylcarnitine (C18-1)			-	increased	< 0.05		-
Hydroxystearoylcarnitine (C18-OH)	< 0.0001	increased	< 0.05	increased	< 0.0001	increased	< 0.05
Decadienoylcarnitine (C10-2)	< 0.0001	increased	< 0.001		-		< 0.0001
Hydroxyhexadecanoylcarnitine (C16-OH)	< 0.05	increased	< 0.01		-		-
Decenoylcarnitine (C10-1)	< 0.001		-		-	decreased	< 0.0001

Based on the received results, it may be concluded, that concentration levels of glutamine, C5, C18-OH, C18-1, C16-1, C16 and Anthranillic acid are significantly elevated along the coronary artery progression (non-CVD → IHD → AMI), whereas levels of idole-3-propionic acid are significantly decreased.

At the same time, plasma concentration of SDMA, acetylcholine, DMG, serotonin, phenylalanine, C0, C6-DC and C14-OH were found to be significantly elevated in patients with IHD and AMI in comparison with patients without CVD.

Significant changes in plasma levels of 3-aminoisobutiric acid, choline, NMMA, valine, leucine, isoleucine, kynurenic acid, kynurenine, cystathionine, C10-2, C16-OH and cystathionine were found in IHD patients in comparison to Non-CVD and AMI subjects. In contrary, concentration of asparagine was significantly decreased in patients with IHD. At the same time, blood samples of AMI patients were characterized by the significantly elevated levels of VMA, biopterin, methionine sulfoxide, proline and tryptophol, as well as by significantly decreased levels of 3-hydroxykynurenine, aspartic acid, norepinephrine, xanthurenic acid, methionine, citrulline, glycine and C10-1 compared to non-CVD and IHD subjects.

Graphical interpretation of the received results after min-max normalization is presented in Fig. 4 (A-E) (A – amino acid profiling; B – acylcarnitine profiling; C – metabolites of NO/urea cycle, neurotransmitters and neuromodulators; D – Tryptophan metabolism intermediates, E – known ratios of endogenous metabolites).

3.4 Weighted correlation network analysis

Additionally, weighted correlation network analysis was performed, serving for identification of the new relationships between the profiled metabolites and quantified clinical factors. Thus, it explains functional metabolic modules of the measured metabolites in plasma of non-CVD, IHD and AMI subjects (Fig. 5A, B and C, respectively), as well, as how they correlate with anthropometric and clinical factors. Additional information, regarding characteristics of the received networks, is presented in the supplementary material (Supplementary material, table S2).

According to the received results, three specific modules (I, II, III), among which only the first one, containing acylcarnitines, almost remained unchanged. In each module it was possible to identify main hub metabolites.

Moreover, based on the differences in the constructed networks, there were found new significant ratios of the metabolites. Among 23 established significantly altered metabolite ratios 14 ratios between non-CVD vs AMI and 17 ratios between IHD vs AMI were found. 9 ratios between non-CVD vs AMI and IHD vs AMI and 2 ratios between non-CVD vs IHD vs AMI were coincided (Table 4). These ratios may further be utilized, as new potential biomarkers of IHD and AMI.

Table 4
Significantly altered metabolite ratios, based on the conducted weighted correlation network analysis

Ratio	p-value non-CVD – IHD	p-value non-CVD – AMI	p-value IHD – AMI	p-value Kruskal
Biopterin: methionine sulfoxide		< 0.00001	< 0.00001	< 0.00001
Anthranilic acid: Aspartic acid	< 0.001	< 0.00001	< 0.001	< 0.00001
3OH anthranilic acid: Tryptophol		< 0.00001	< 0.00001	< 0.00001
Anthranilic acid: Kynurenine		< 0.00001	< 0.00001	< 0.00001
GSG	< 0.01	< 0.00001	< 0.0001	< 0.00001
3OH anthranilic acid: Tryptophol		< 0.00001		< 0.00001
Tryptophan: 5OH-Tryptophan	< 0.0001	< 0.0001		< 0.00001
C18: Indole-3-propionic acid		< 0.0001	< 0.01	< 0.0001
Aspartic acid: Biopterin		< 0.001	< 0.001	< 0.0001
Anthranilic acid: Methionine		< 0.001	< 0.001	< 0.0001
ADMA: NNMA			< 0.0001	< 0.001
Kynurenine: Tryptophan	< 0.01		< 0.05	< 0.001
NMMA: Indole-3-carboxaldehyde	< 0.05		< 0.001	< 0.001
Biopterin: Metanephrine		< 0.01	< 0.01	< 0.01
Citrulline: Ornithine		< 0.01		< 0.01
C12:C14-OH	< 0.001	< 0.01		< 0.01
3-OH-Kynurenine: Kynurenine			< 0.01	< 0.01
Arginine: ADMA		< 0.001		< 0.01
Norepinephrine Metanephrine			< 0.01	< 0.01
Asparagine: Histidine			< 0.01	< 0.05
ADMA: Proline			< 0.05	< 0.05
Fischer			< 0.01	< 0.05
Aspartic acid: Methionine	< 0.01			< 0.05
n = 23	n = 7	n = 14	n = 17	

Graphical interpretation of the selected most significant ratios is presented in Fig. 6.

4. Discussion

4.1 Significant changes in concentration levels of the plasma metabolites in Non-CVD subjects, patients with IHD and patients with AMI

The presented data is the continuing of our previous study of plasma metabolites in IHD patients [7]. Based on the conducted metabolomic profiling, it may be revealed, that significant alterations in concentration levels of kynurenine, kynurenic acid, as well as kynurenine / tryptophan ratio, were found only in IHD group, whereas in AMI patients its levels did not differ from the plasma levels of non-CVD subjects. At the same time concentration of xanthurenic acid and 3-OH-kynurenine did not differ among the non-CVD and IHD subjects, but was significantly decreased in patients with AMI. Overall, the above mentioned metabolites are related to the kynurenine metabolic pathway (KMP) – main route of the tryptophan catabolism. KMP is presumably responsible for cellular energetic homeostasis, being the principal regulator of the immune system [8]. Therefore, KMP may reflect the inflammation processes being occurred in the body during the disease. Numerous studies suggest the application of KMP metabolites, as potential prognostic biomarkers of IHD [9]. In this regard, significant changes in concentration levels of key KMP intermediates (kynurenine and kynurenic acid) may be related to the activation of the indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) enzymes. These enzymes are mainly induced by pro-inflammatory stimuli and T-cell cytokines during the pro-inflammatory states and immune stress associated with IHD, thus suggesting, that the perturbations in IHD are mostly related to the activation of kynurenine degradation to the formation of kynurenic acid. At the same time, it may be concluded, that completely different metabolic pathways are activated in patients with myocardial infarction, presumably resulting in the decrease of the enzymes responsible for production of xanthurenic acid and 3-OH kynurenine – metabolites of an alternative to kynurenine degradation pathway. Xanthurenic acid is a well-known vasorelaxant, thus its decreased levels may indicate the need for prescription of medicines containing xanthurenic acid in patients with AMI [10]. In this case, the mechanism of action of xanthurenic acid is based on its influence on endothelial levels of nitric oxide, thus causing hypertension.

Plasma serotonin levels were found to be significantly altered in patients, suffering from IHD and AMI in comparison to non-CVD subjects. It should be mentioned, that serotonin itself possesses numerous complex effects, acting as peripheral hormone and neurotransmitter [11]. Prevalent part of serotonin is deposited in dense granules of platelets, being released in the bloodstream, as the result of its activation by thrombus formation in coronary arteries. As a result, it may lead to aggregation of platelets and constriction of coronary smooth cells [12, 13].

Several amino acids were significantly changed in IHD and AMI patients in comparison to the non-CVD subjects. However, these alterations were not linear. For example, there was found significant elevation in concentration levels of branch-chained amino acids (BCAA) metabolites in IHD patients, whereas in AMI group their level was equal to the concentration in the non-CVD group.

Overall, amino acids play a crucial role in the energy metabolism and are directly involved in various metabolic pathways, including Krebs cycle and gluconeogenesis, therefore significant changes in their concentration levels may reflect disturbances in the energy metabolism. Due to the relatively large amount of significantly altered metabolites in IHD and AMI groups of patients, application of an amino acid metabolic profiling may be used as a novel biomarker diagnostic and prognostic panel for identification of IHD and AMI patients.

Endogenous neurotransmitter norepinephrine was significantly decreased in patients with AMI, whereas its end-product – vanillylmandelic acid (VMA) – significantly increased. Plasma norepinephrine is mainly derived from sympathetic nerves, thus indicating activity of the sympathetic nervous system. Through specific binding to α_1 and α_2-adrenoreceptors it acts as vasoconstrictor. Significant decrease in concentration of plasma norepinephrine may be associated with increase of its cellular uptake in AMI patients. In this case, such cellular uptake leads to its

deamination with the formation of methoxyhydroxyphenylglycol (MHPG), followed by its oxidation to VMA [14]. According to [15] 94% of VMA is synthesized in liver, among which in 87% it represents the results of hepatic extraction and metabolism of 3-Methoxy-4-hydroxyphenylglycol (MHPG) and DHPG. In this regard, we may hypothesize, that patients from the AMI group had more activated norepinephrine cellular uptake, that resulted in extra accumulation of VMA. However, for verification of this hypothesis it is also needed to quantify intermediate products of norepinephrine, including MHPG and dihydroxyphenylglycol.

Acetylcholine represents another neurotransmitter which is released in response to innervation of the blood vessels by the parasympathetic cholinergic or sympathetic cholinergic nerves. Binding of acetylcholine with the muscarinic M3 receptor leads to the release of NO, resulting in smooth muscle relaxation. However, it is known, that in case of NO absence, M3 receptor action is opposite, resulting in constriction of the smooth muscles [16]. In the presented study we have identified the significant alteration in the concentration levels of acetylcholine in patients with IHD and AMI, therefore suggesting the lack of NO in patients with the considered CVD disorders. At the same time, acetylcholine precursor – choline – was significantly elevated only in IHD patients, while in AMI patients its levels became equal to those from the non-CVD group. Elevated choline levels are the markers of unfavorable cardiovascular risk profile and CVD incidences [17].

Intermediates of the homocysteine-methionine cycle are directly connected with choline through its derivative – betaine, and are responsible for amino acid metabolism and cellular methylation. In the presented study we have identified a strongly impaired regulation of this cycle, including significantly increased levels of cystathionine and decreased levels of methionine in the AMI patients. Interestingly, that the methionine derivative was significantly altered in AMI patients, that may be the result of methionine oxidation. Elevated levels of cystathionine identified in the presented study are known to be linked to oxidative damage and impaired endothelial function [18].

Dimethylglycine, that was significantly altered in IHD and AMI patients, is usually produced from betaine upon remethylation of homocysteine to methionine [19]. Previously, numerous studies identified the dimethylglycine as a major risk marker of myocardial infarction in patients with suspected or established coronary heart disease [20]. At the same time, the decreased levels of glycine may confirm the accumulation of dimethylglycine in plasma.

The presented study identified, that short-chain and long-chain acylcarnitines were significantly increased in both IHD and AMI groups. Acylcarnitines play a significant role in the myocardial metabolism, being mainly responsible for mitochondrial β-oxidation of long-chain fatty acids, and, therefore, for energy production. Numerous studies have linked disturbance in blood acylcarnitines to various cardiovascular disorders [3, 21–23]. Deprivation of oxygen and nutrients in myocardium during IHD and AMI reveals breakdown of fatty acids. In this case, elevated levels of acylcarnitines reflect disbalance in mitochondrial function and oxidative stress.

Levels of symmetric dimethylarginine (SDMA) were significantly increased in IHD and AMI patients. SDMA is known as an endogenous inhibitor of NO-synthase, therefore, its elevated levels possess extra inhibition of NO production. At the same time, increased levels of biopterin in the AMI patients may underline an alternative NO-generating pathway in patients after AMI, which is presumably caused by the compensatory mechanisms in the body, due to the acute cardiac event. In general, NO is one of the key vasodilators in the body, which is responsible for blood pressure control, therefore, its inhibition may lead to endothelial dysfunction and oxidative stress.

Citrulline, an intermediate of the urea cycle, was significantly decreased in patients with myocardial infarction in comparison to non-CVD subjects and IHD patients. Recyclization of citrulline to arginine is one of the principle steps of the endothelial NO synthesis. In this case decreased levels of citrulline may contribute to the impaired NO production in AMI patients.

In comparison to non-CVD individuals, patients with AMI had increased plasma levels of Carnitine, Isovalerylcarnitine, long-chain acylcarnitines (Palmitoylcarnitine, Palmitoleyl carnitine, Oleoylcarnitine, Hydroxystearoylcarnitine), amino acids (phenylalanine, glutamine), biopterin, symmetric dimethylarginine, tryptophan metabolism derivatives (Tryptophol, Serotonin, Anthranilic acid), Vanillylmandelic acid, Methionine sulfoxide, acetylcholine, and Dimethylglycine. At the same time, plasma concentration levels of glycine, aspartic acid and indole-3-propionic acid were significantly decreased. These data correspond to our previously obtained results [7].

In comparison to patients with IHD, patients with AMI had significant increased plasma levels of Dimethylglycine, Vanillylmandelic acid, Tryptophol, biopterin, glutamine, proline, asparagine, Isovalerylcarnitine and Hydroxystearoylcarnitine. In contrary, plasma levels of Norepinephirne, Xanthurenic acid, 3-Hydroxykynurenine, Citrulline, valine, leucine, N-methylmalonamic acid, Aspartic acid, Methionine, Glycine and Decenoylcarnitine were significantly decreased.

Figure 7 summarizes metabolic pathways, affected by the identified significantly altered metabolites.

4.3 Differences in the weighted correlation network analysis of the considered metabolites

The above presented pathway analysis and interpretation of the metabolomic profiling were based on the mapping of the identified significantly changed metabolites into preliminary defined pathways, obtained from metabolic databases, such as KEGG [24] or MetaCyc [25]. At the same time, weighted correlation network analysis represents an alternative powerful tool for identification of systemic metabolic changes, that are often undetectable, when using solely changes in metabolite levels and database pathway information [26]. In the presented study, there was utilized a DSPC algorithm, the regularized approach mainly created for handling high dimensional MS-based metabolomic profiling data [27]. Its main principle is based on the application of the de-sparsified graphical lasso model, taking into account, that the amount of true connections among the metabolites is significantly smaller than the utilized sample size.

It was found, that the first module mainly consisting of medium and long chain acylcarnitines was relatively the same in all three considered groups of patients, as well, as the principal nodes. At the same time, short chain acylcarnitines were separated from the module forming short chains presumably containing C5, C3 and C0 metabolites. Also, it should be mentioned, that this module in all groups of patients was connected with VMA, showing unchangeable relations of medium and long chain acylcarnitines with the end-product of the norepinephrine degradation pathway. However, we may conclude, that the number of intra edges of this module in AMI was significantly lower compared to IHD and non-CVD subjects, that may be explained by the alternative activity of these metabolites after AMI.

The second module is mainly consisted of kynurenines. The key nodes were Indole-3-acrylic acid, kynurenine and Indole-3-carboxaldehyde in non-CVD, IHD and AMI groups, respectively. In non-CVD group the kynurenine module showed strong relation with choline, SDMA and ADMA, whereas in patients with IHD and AMI these connections were absent. In AMI patients the tryptophan pathway metabolites were “diveded” into two parts: one connected with amino acids, and the second – with neopterine.

The third module is primarily comprised of amino acids. Among these metabolites it should be mentioned, that strong correlation between BCAA was relatively the same in all three considered groups of patients. Moreover, in non-CVD and IHD groups the amino acid module is also containing the relations with NO-cycle intermediates.

4.4 Advantages and limitations of the study

The main advantage of the study is that the presented approach provides new insights into the development of AMI from the metabolic point of view. There were found new significant ratios of the metabolites, which may further be utilized as new potential biomarkers of AMI.

This study is not without limitations. The main of it is the necessity of confirmation the results obtained, which need a large number of patients and future studies.

Conclusions

In conclusion, our comprehensive study has shed light on the intricate metabolic changes associated with AMI and IHD, offering valuable insights into the underlying metabolic dysregulations. Through targeted metabolomic profiling and statistical analyses, we identified significant alterations in metabolite levels between AMI/IHD patients and non-CVD subjects. These changes encompassed various metabolic pathways, including amino acids, acylcarnitines, and tryptophan catabolism.

Additionally, our study went beyond traditional metabolomic analyses by employing Weighted Correlation Network Analysis (WGCNA). This network-based approach unveiled subtle, but crucial alterations in metabolic modules among patient groups, enhancing our understanding of systemic metabolic changes.

We hypothesize, that this approach will be useful in screening of patients with IHD and AMI risk developing.

In summary, our research contributes to the growing body of knowledge surrounding IHD and AMI, that urges to emphasize the significance of metabolic profiling and network-based analyses in unraveling the intricacies of cardiovascular diseases. These insights may pave the way for more effective diagnostic and therapeutic strategies in the future, ultimately improving patient care and outcomes.

Declarations

Data availability

All data generated and analyzed during this study are included in this published article and its Supplementary Information file.

Author contributions

S.S.M.: Conceptualization, Writing—review & editing; E.A.P.: Writing—review & editing, Supervision; Y.A.R.: Data curation, Supervision; T.O.P.: Data curation, Supervision; S.V.I.: Writing—original draft; V.V.B.: Conceptualization; S.L.K.: Data collection, Patient supervision; G.I.S.: Data collection, Patient supervision; Zh.Yu.Ch.: Data collection, Patient supervision; Y.A.L.: Data collection, Patient supervision; I.M.K.: Data collection, Patient supervision; A.G.K.: Data collection, Patient supervision; K.M.S.: Writing—original draft, Bioinformatics; P.A.M.: Laboratory analysis; N.E.M.: Laboratory analysis; S.A.A.: Writing—original draft.

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Competing interests

The authors declare no competing interests.

Correspondence and requests for materials should be addressed to S.S.M.

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Figures

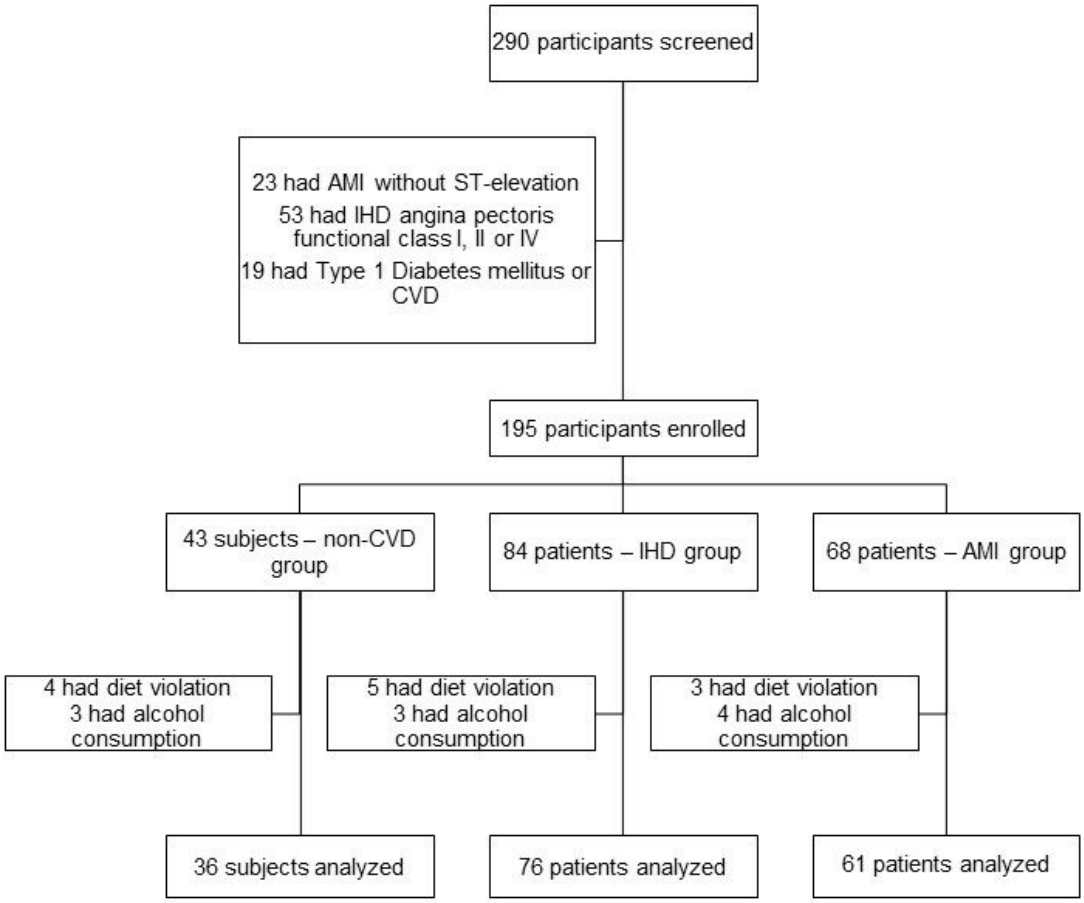

Figure 1

Trial profile

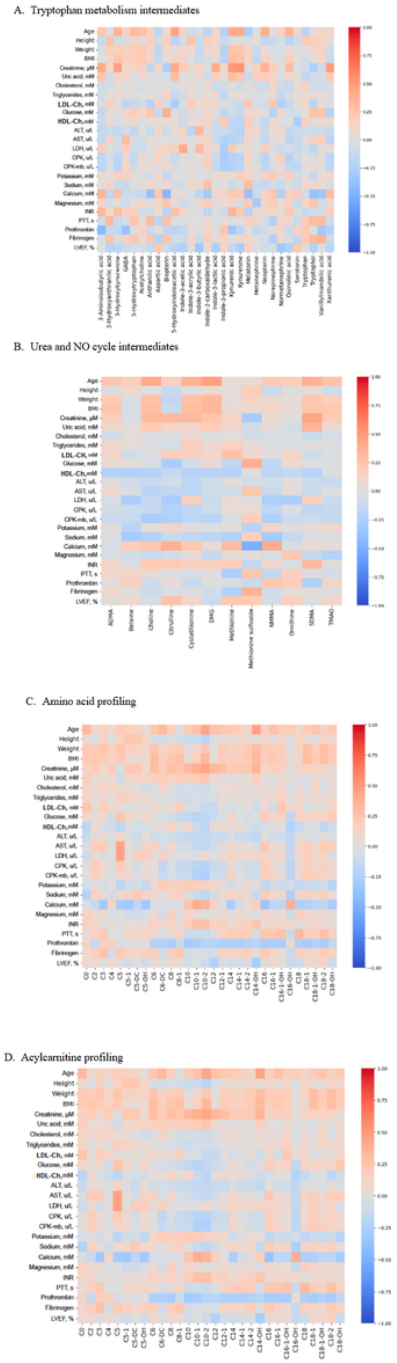

Figure 2

Heatmap correlation matrices between plasma metabolites and results of the biochemical analysis

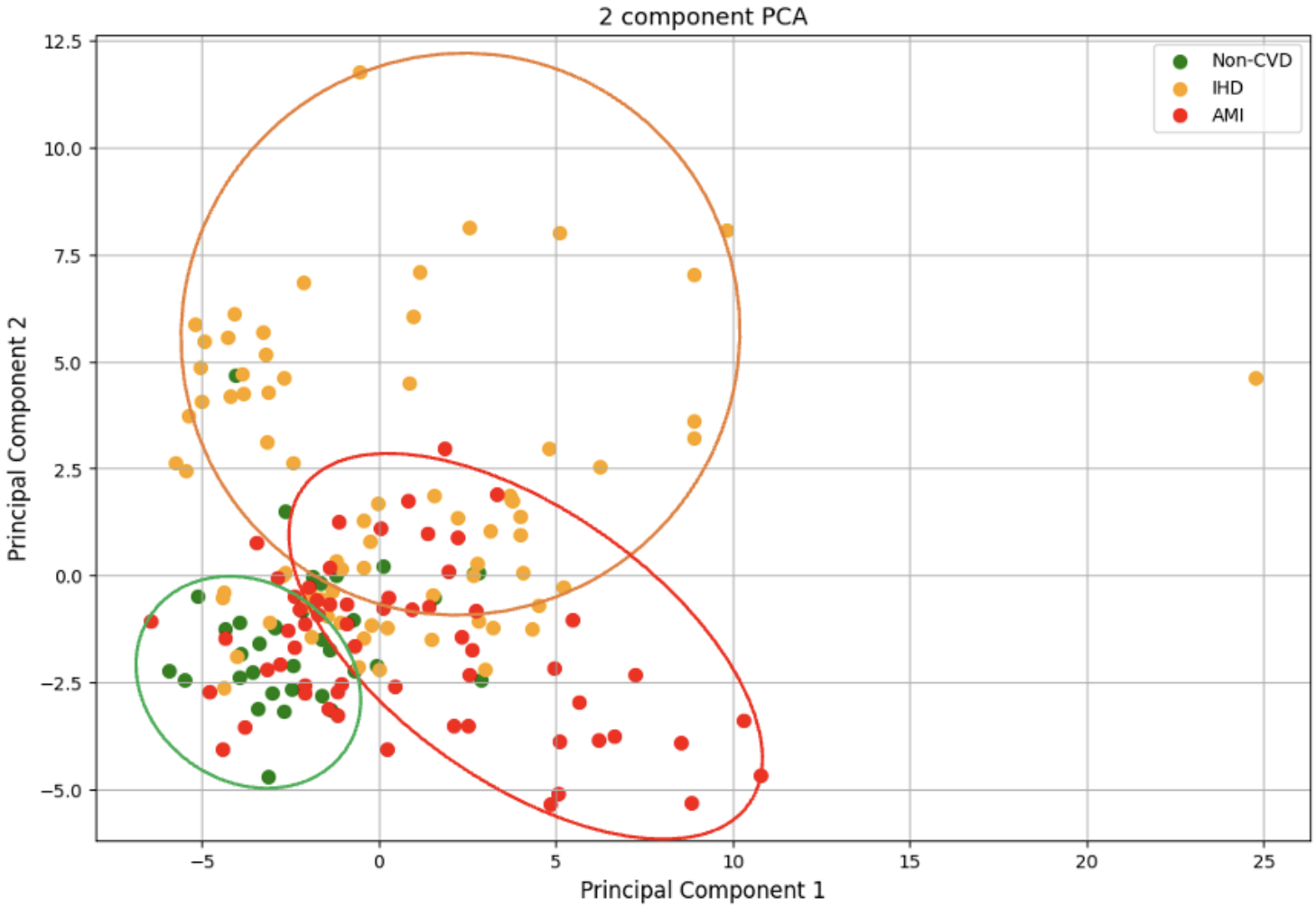

Figure 3

PCA analysis of the analyzed samples

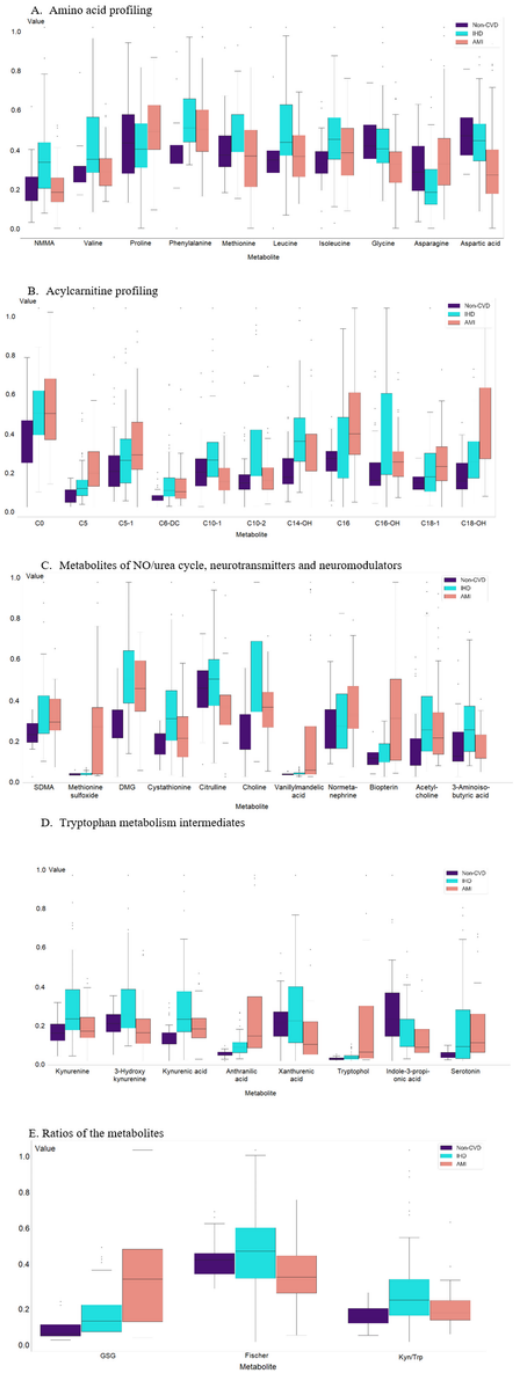

Figure 4

Horizontal bar-charts of the significantly changed metabolites among the studied groups of patients

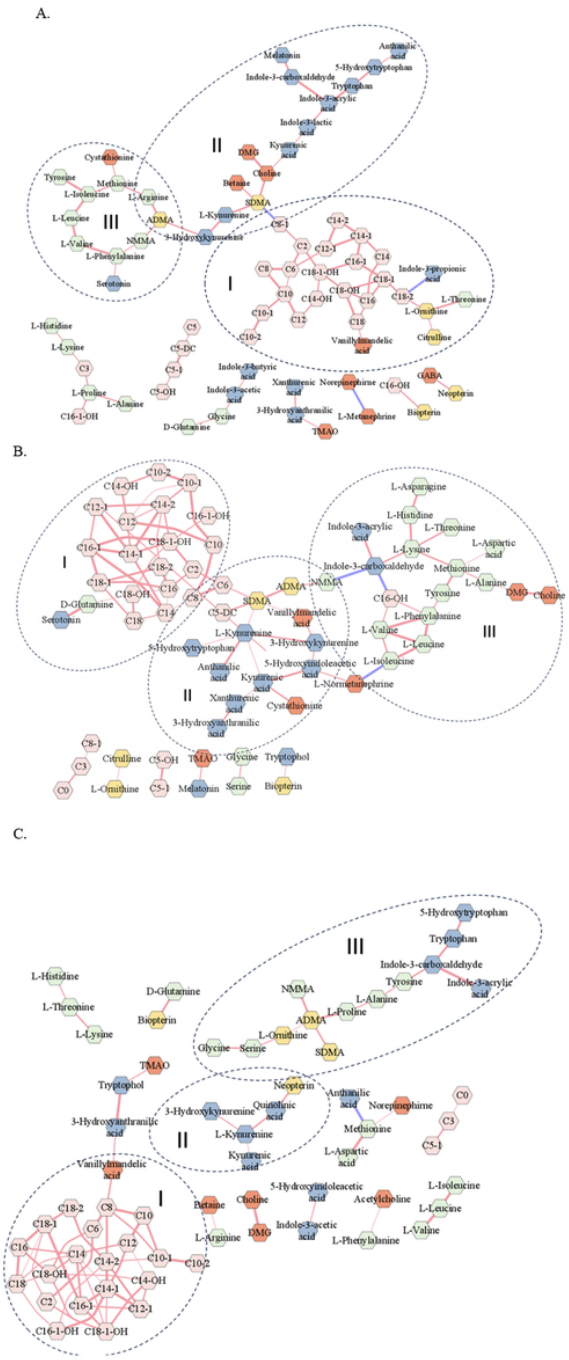

Figure 5

Weighted correlation networks in: A – non-CVD group; B – IHD group; C – AMI group. I, II, III – metabolites' clusters.

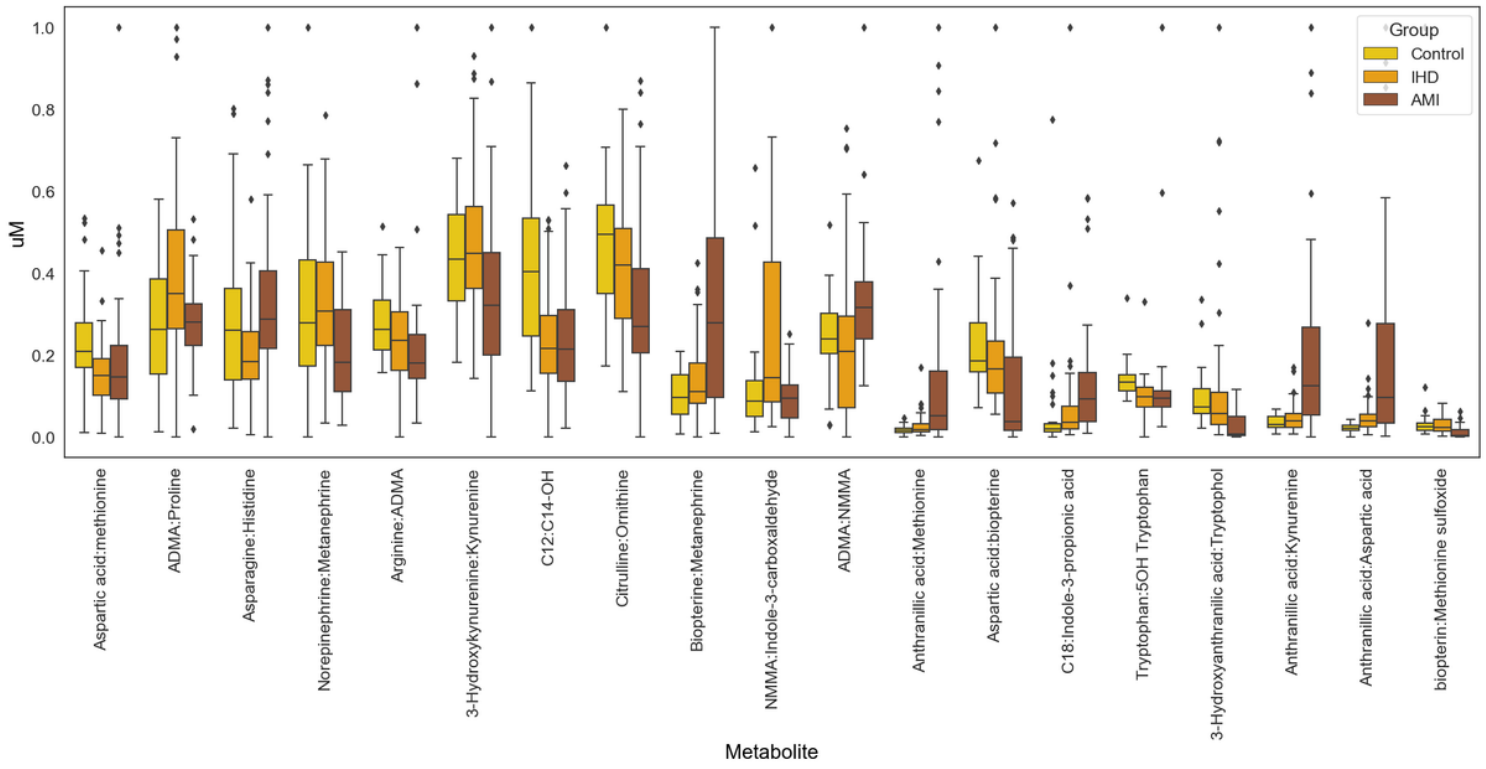

Figure 6

Horizontal bar charts of the significantly changed ratios of the metabolites, based on the results of the weighted correlation network analysis.

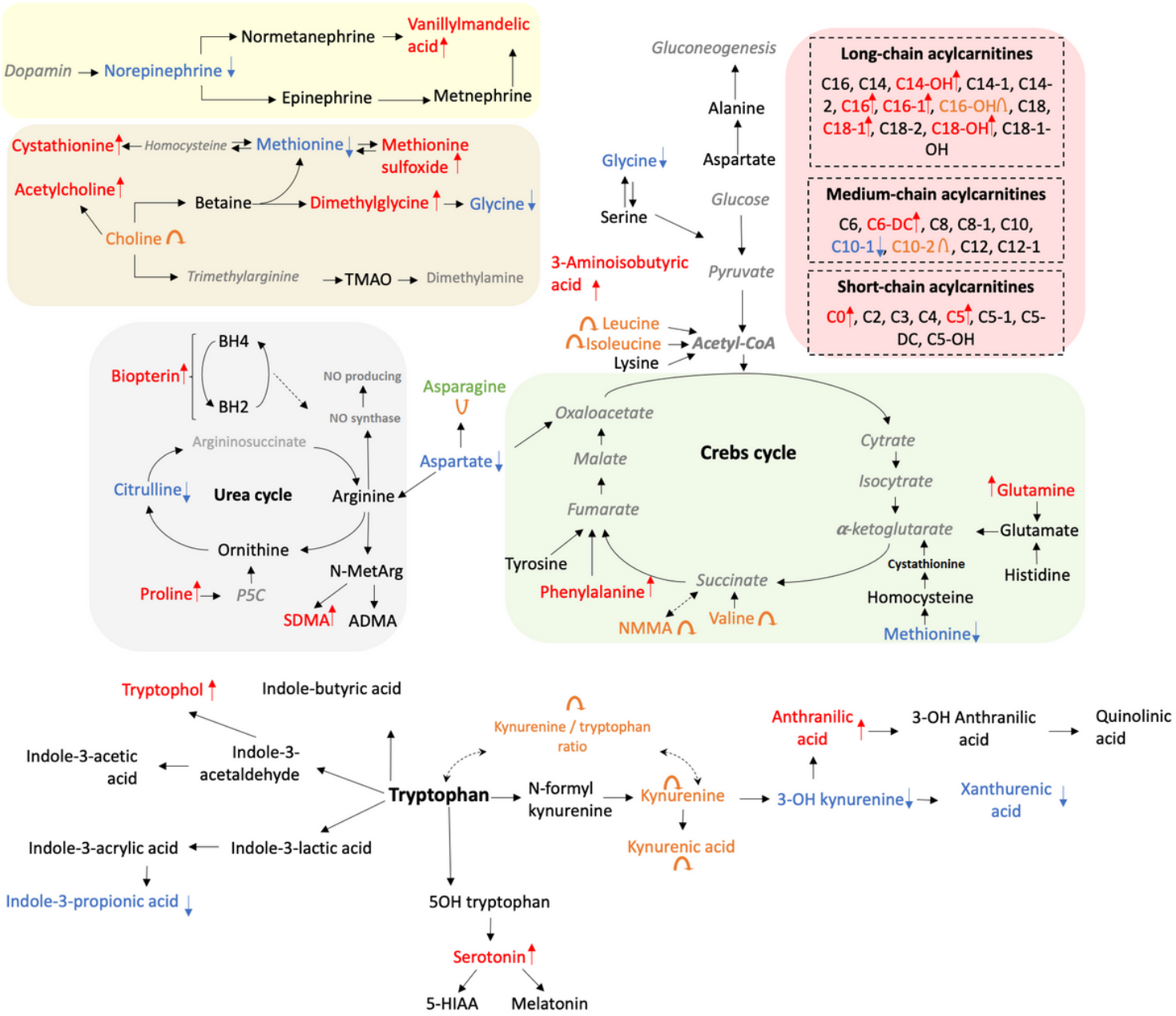

Figure 7

Significantly changed metabolites and metabolic pathways in the AMI pathogenesis.

Supplementary Files

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- MarkinS.S.Supplementary2.docx