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Metabolomic Profiling of Patients with Alcoholic Liver Disease and Non-Alcoholic Fatty Liver Disease Using Principal Component Analysis

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Aim: to investigate the metabolomic profile of patients with alcoholic liver disease and non-alcoholic fatty liver disease.
Materials and methods. The present study included patients diagnosed with non-alcoholic fatty liver disease (NAFLD) (n = 44), patients diagnosed with alcoholic liver disease (ALD) (n = 40) and 14 healthy volunteers. The level of metabolites in the blood serum were determined via high-performance liquid chromatography and tandem mass spectrometry.
Results. In this study, a cross-sectional targeted metabolomic analysis of 96 serum metabolites was performed in patients. Statistical analysis using the principal component method identified six main factors, comprising metabolites from various metabolic pathways. Comparative analysis between patient groups and the control group revealed statistically significant differences in the metabolic activity of individual factors, collectively reflecting alterations in the metabolomic profile. Levels of acylcarnitines, uridine, metanephrine, asymmetric and total dimethylarginine were elevated in patients with NAFLD and ALD compared to the control group. Carnitine, short chain acylcarnitines, valine, leucine, lysine, and alanine were significantly higher in patients with NAFLD compared to those with ALD. In contrast, levels of tyrosine, epinephrine, and methionine were significantly increased in ALD patients compared to both NAFLD patients and healthy volunteers. Among patients with liver cirrhosis (both ALD and NAFLD), there was a noticeable trend toward altered metabolic activity of factors correlating with liver failure indicators and the FIB-4 index. As liver cirrhosis progressed, statistically significant changes in metabolite levels were observed across Child — Pugh classes, taking into account hypocoagulation, hypoalbuminemia, hyperbilirubinemia, the presence of ascites, and hepatic encephalopathy.
Keywords: metabolomic profile, principal component analysis, alcoholic liver disease, non-alcoholic fatty liver disease, liver cirrhosis
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Определение метаболомного профиля пациентов с алкогольной болезнью печени и неалкогольной жировой болезнью печени методом главных компонент

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Цель исследования: изучение метаболического профиля пациентов с алкогольной болезнью печени и неалкогольной жировой болезнью печени.
Материалы и методы. В настоящем исследовании приняли участие пациенты с диагнозом «неалкогольная жировая болезнь печени» (НАЖБП) (n = 44), пациенты с диагнозом «алкогольная болезнь печени» (АБП) (n = 40) и 14 здоровых добровольцев. Уровень метаболитов в сыворотке крови определяли с помощью высокоэффективной жидкостной хроматографии и тандемной масс-спектрометрии.

Результаты. Был проведен одномоментный целевой метаболомный анализ 96 метаболитов сыворотки крови пациентов. Статистический анализ с использованием метода главных компонент выявил шесть основных факторов, включающих метаболиты из различных метаболических путей. Сравнительный анализ между группами пациентов и контрольной группой выявил статистически значимые различия в метаболической активности отдельных факторов, в совокупности отражающие изменения метаболомного профиля. У пациентов с НАЖБП и АБП по сравнению с контрольной группой были повышены уровни ацилкарнитинов, уридина, метанефрина, асимметричного и общего диметиларгинина. Напротив, уровни тирозина, эпинефрина и метионина были значительно повышены у пациентов с АБП по сравнению как с пациентами с НАЖБП, так и со здоровыми добровольцами. Среди пациентов с циррозом печени (как с АБП, так и с НАЖБП) наблюдалась заметная тенденция к изменению метаболической активности факторов, коррелирующих с показателями печеночной недостаточности и индексом FIB-4. По мере прогрессирования цирроза печени на разных стадиях заболевания, согласно классификации по шкале Чайлда — Пью, наблюдались статистически значимые изменения уровней метаболитов с учетом наличия гипокоагуляции, гипоальбуминемии, гипербилирубинемии, асцита и печеночной энцефалопатии.

Ключевые слова: метаболомный профиль, метод главных компонент, алкогольная болезнь печени, неалкогольная жировая болезнь печени, цирроз печени

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Introduction

Advancing our understanding of disease development and progression is impossible without improving research methodologies. One of such emerging scientific approaches is metabolomics, a branch of biochemistry designed to study the concentrations of small molecules (metabolites) in biological matrices [1]. Currently, more than 220,000 active metabolites involved in various biochemical processes in the human body have been described. The maintenance of homeostasis is largely depends on the regulation of anabolic and catabolic processes involving highly active small molecules such as amino acids, lipids, sugars, and organic acids. Alterations in the metabolome occur in response to various endogenous processes within the body and can more accurately characterize specific biological phenotypes.

In clinical medicine, metabolomic analysis enables a more precise description of biochemical changes associated with diseases of various organs and systems. Current research is focused on identifying new, more accurate biomarkers that can enhance disease diagnosis and prevention, as well as potential molecular targets for therapeutic intervention. To date, several metabolomic markers have already been identified as predictors of diabetes mellitus, cardiovascular complications, and certain types of cancer [2–4].

The modern predominant statistical method used in metabolomics is factor analysis of the correlation matrix via principal component analysis. In the course of this analysis, it becomes possible to identify complex factors (components) that incorporate key variables and provide the most comprehensive explanation of observed correlations. It can also reveal hidden interconnections between biochemical processes occurring in the human body under various pathological conditions.

The aim of the present study was to investigate the metabolomic profile of patients with alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD).

Materials and methods

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the I.M. Sechenov First Moscow State Medical University (Sechenov University) No. 01-22 dated 01/20/2022, for the period from 02/22/2022 to 02/14/2023. Patients with an established diagnosis of NAFLD were included in the work. The diagnosis was established on the basis of anamnesis, clinical picture and data from laboratory and instrumental studies, as well as the exclusion of other etiological factors of liver damage (diagnosis *per exclusionem*). If alcohol consumption in hepatotoxic doses for > 6 months (> 30 g/day for men and > 20 g/day for women) was detected, a diagnosis of ALD was established.

All patients were screened for the presence of viral hepatitis markers (HBsAg, HBeAg, Anti-HBc-total, Anti-HBe Anti-HBs, Anti-HCV-total, Anti-HAV-IgM, Anti-HAV-IgG), markers of primary biliary cholangitis and autoimmune hepatitis (AMA, ANA, SMA and anti-LKM1), as well

as hemochromatosis (ferritin level, transferrin saturation with iron) and Wilson's disease (ceruloplasmin level, Kayser — Fleischer rings, daily amount of copper in urine, free copper level in blood plasma). Patients also underwent ultrasound examination (US) of abdominal organs and study of alpha-fetoprotein level to exclude neoplasms of the liver and abdominal organs. The type of liver damage (steatohepatitis/cirrhosis) was determined considering the conclusion of the histological examination in combination with clinical data, as well as data from laboratory and instrumental research methods.

The control group consisted of healthy volunteers with no complaints from the gastrointestinal tract, without concomitant diseases of the respiratory, urinary, endocrine, cardiovascular systems and oncological diseases, who came to the clinic for a preventive examination.

Metabolomic analysis

At the first stage, upon the patient's admission to the hospital, blood was drawn from the brachial vein into 2 mL vacuum tubes with the clot activator. The blood samples were centrifuged under standard conditions at room temperature at an acceleration of 3,000 g for 10 minutes. After precipitation, the supernatant, blood serum, was poured into polypropylene Eppendorf tubes. The samples were stored at temperatures below –80 °C until the analysis stage.

In the second stage, solutions of internal metabolite standards were prepared. After thawing, 40 μL of the internal standard mix (IS-mix) was added to 10 μL of the plasma samples in polypropylene Eppendorf tubes. The resulting mixture was evaporated to dryness in a centrifugal vacuum evaporator at a temperature of 40 °C. To the dry residue, a derivatization mixture (a solution of phenylisothiocyanate in pyridine) was added, then the solution was mixed on a vortex for 10 seconds and left at room temperature for 20 minutes. The samples were then evaporated to dryness again in a centrifugal vacuum evaporator at a temperature of 40 °C. To the dry residue, 100 μL of a 5 mM ammonium acetate solution in methanol was added. The samples were mixed on a shaker for 30 minutes. To the plasma samples and laboratory plasma samples, 100 μL of deionized water was added, and to the standard solutions (Cal1-6, QCs), 100 μL of a 0.1 PBS solution was added. The resulting solutions were mixed in a vortex for 10 seconds and centrifuged at 14,900 g and a temperature of 20 °C for 10 minutes. A supernatant volume of 150 μL was transferred to a polypropylene microvial for subsequent chromatography–mass spectrometry analysis.

In the course of high-performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS), the identification of compounds in the sample was conducted based on two characteristics, retention time and characteristic mass spectrometric transitions. The chromatographic separation of substances was performed using gradient elution. Mass spectrometric detection was performed in the multiple reaction monitoring (MRM) mode.

Parameters of the high-performance liquid chromatography: analytical column for HPLC — ACQUITY UPLC BEH C18, 2.1 × 50 mm; particle size — 1.7 μm; pre-column filter — ACQUITY UPLC BEH C18; the temperature control of the analytical column — 40 °C; the flow rate of the mobile phase — 0.50 mL/min. The composition of the mobile phase: phase a — 0.1 % formic acid solution in deionized water; phase b — 100 % acetonitrile for chromatography. Sample injection volume — 5 μL. Total analysis time — 5 minutes.

Parameters of the tandem quadrupole mass spectrometric detector: type of ionization — electrospray with heated nebulizing gas flow (Agilent Jet Stream); gas temperature — 300 °C; gas flow — 11 L/min; nebulizer — 35 psi; sheath gas temperature — 300 °C; sheath gas flow — 11 L/min; capillary voltage (positive ionization) — 3.5 kV; capillary voltage (negative ionization) — 3.5 kV. The analysis was carried out using the dynamic multiple reaction method (dynamic MRM), the duration of the dynamic analysis cycle was 600 ms.

After the analysis was completed, the obtained data were processed using the MassHunter software with the construction of a calibration curve. The result of the quantitative determination of the samples was outputted in the form of a general Excel file containing sample numbers and analyte concentrations. A total of 96 different serum metabolites were identified in the metabolomics analysis.

Statistical analysis

Statistical data processing was performed using application software. The Kolmogorov — Smirnov criterion with Lilliefors correction was used to check the distribution of quantitative indicators for normality. Variables with a non-normal distribution were described as median and interquartile range — *Me* [Q1; Q3]. Qualitative data were presented as absolute and relative values — *n* (%). Comparisons of two independent groups by quantitative variables were assessed using the non-parametric Mann — Whitney U criterion, and for three or more groups — the Kruskal — Wallis test. The significance of the differences between the groups in terms of qualitative indicators was evaluated

using the χ^2 Pearson criterion. The correlation between the quantitative variables was established using Spearman's rank correlation coefficient. The strength of the correlation was evaluated according to Chaddock's scale. The significance level for hypothesis testing was set at $p < 0.05$. To correct for type 1 error in multiple pairwise comparisons, the Bonferroni correction was applied.

To identify and interpret the hidden structure in the set of studied indicators, factor analysis using the Principal Component Analysis (PCA) method with orthogonal varimax rotation was performed. Prior to analysis, the adequacy of the data was assessed using the Kaiser — Meyer — Olkin (KMO) criterion and Bartlett's test of sphericity, confirming the presence of sufficiently strong relationships in the correlation matrix. In the first step, principal components were extracted from the total set of variables and ordered in descending order of explained variance; then, based on visual analysis of the "scree plot" and the Kaiser criterion (eigenvalues > 1), the optimal number of factors was determined. After obtaining the final factor structure, the loadings of each variable on the factors were analyzed to determine which metabolites and parameters formed coherent clusters reflecting underlying latent processes or common metabolic pathways.

Results

Data from 98 individuals were analyzed: 44 patients with a confirmed diagnosis of NAFLD, 40 patients with ALD diagnosis and 14 healthy volunteers. The main clinical and laboratory characteristics of the studied groups are presented in Table 1.

All patients were comparable by gender ($p > 0.05$), the average age in the control group was slightly lower ($p = 0.0001$), however, no statistically significant differences were found between the ALD and NAFLD patient groups ($p = 0.30$). Patients with NAFLD showed a significant increase in BMI ($p < 0.0001$).

In the biochemical blood analysis, patients with ALD had pronounced hyperbilirubinemia due to both total and direct bilirubin fractions ($p < 0.0001$ and $p = 0.003$, respectively), and compared to the control group, elevated markers of cholestasis were observed — gamma-glutamyl transferase ($p < 0.0001$) and alkaline phosphatase ($p = 0.001$).

In the lipid profile analysis, patients with NAFLD had a significant increase in very low-density lipoproteins (VLDL) compared to the ALD group ($p < 0.0001$) and the control group ($p = 0.0024$). No statistically significant differences were found between patient groups in other lipid fractions.

Table 1. Characteristics of clinical and laboratory data of the study groups
Таблица 1. Характеристика клинико-лабораторных данных исследуемых групп

Variable ***Переменная***		**ALD** ***АБП*** **(*n* = 40)**	**NAFLD** ***НАЖБП*** **(*n* = 44)**	**Control group** ***Группа контроля*** **(*n* = 14)**	***p***
Demographic data ***Демографические данные***					
Gender / *Пол* *n* (%)	Males / *Мужчины*	22 (55.00 %)	16 (36.36 %)	3 (21.43 %)	0.055
	Males / *Женщины*	18 (45.00 %)	28 (63.64 %)	11 (78.57 %)	
Age, years / *Возраст, годы*		51.50 [41.75; 60.25]	57.00 [48.00; 63.50]	40.50 [29.75; 44.75]	0.0001
Laboratory parameters ***Лабораторные показатели***					
Platelets, $\times 10^9$/L *Тромбоциты, $\times 10^9$/л*		163.50 [96.25; 265.00]	237.00 [192.75; 265.00]	307.50 [253.25; 369.50]	0.0001
White blood cells, $\times 10^9$/L *Лейкоциты, $\times 10^9$/л*		5.31 [4.21; 6.93]	6.05 [4.94; 7.42]	6.01 [5.08; 7.68]	0.258
Red blood cells, $\times 10^{12}$/L *Эритроциты, $\times 10^{12}$/л*		4.01 [3.39; 4.65]	4.68 [4.46; 5.03]	4.54 [4.33; 4.79]	< 0.0001
Immunoglobulin A, g/L *Иммуноглобулин А, г/л*		5.51 [3.42; 7.51]	2.66 [2.21; 3.33]	2.53 [2.03; 3.18]	< 0.0001
Immunoglobulin M, g/L *Иммуноглобулин М, г/л*		1.76 [1.26; 2.96]	1.01 [0.75; 1.36]	1.38 [1.31; 1.52]	< 0.0001
Immunoglobulin G, g/L *Иммуноглобулин G, г/л*		15.55 [13.26; 18.98]	11.13 [9.13; 13.23]	11.52 [10.81; 13.03]	< 0.0001

End of Table 1. Characteristics of clinical and laboratory data of the study groups
Окончание таблицы 1. Характеристика клинико-лабораторных данных исследуемых групп

Variable ***Переменная***	**ALD** ***АБП*** **(*n* = 40)**	**NAFLD** ***НАЖБП*** **(*n* = 44)**	**Control group** ***Группа контроля*** **(*n* = 14)**	***p***
Serum total bilirubin, μmol/L *Билирубин общий, мкмоль/л*	29.45 [17.62; 47.53]	12.55 [10.10; 18.92]	10.40 [9.57; 12.65]	< 0.0001
Serum direct bilirubin, μmol/L *Билирубин прямой, мкмоль/л*	9.70 [4.40; 20.06]	2.40 [1.70; 3.30]	2.55 [2.22; 2.88]	< 0.0001
Alanine aminotransferase, U/L *Аланинаминотрансфераза, Ед./л*	26.00 [20.25; 52.25]	34.00 [28.00; 51.75]	17.00 [11.00; 21.00]	0.0004
Aspartate aminotransferase, U/L *Аспартатаминотрансфераза, Ед./л*	55.00 [31.00; 77.50]	26.50 [23.00; 42.00]	20.00 [17.00; 23.00]	< 0.0001
Gamma-glutamyl transferase, U/L *Гамма-глутамилтрансфераза, Ед./л*	97.50 [50.50; 312.00]	35.50 [20.75; 60.25]	14.50 [13.00; 33.25]	< 0.0001
Alkaline phosphatase, U/L *Щелочная фосфатаза, Ед./л*	137.00 [97.00; 263.25]	102.50 [72.75; 169.75]	75.00 [51.00; 104.69]	0.0008
Serum albumin, g/L *Альбумин, г/л*	37.35 [34.00; 43.48]	44.95 [42.92; 46.00]	44.90 [43.47; 46.62]	< 0.0001
Serum total protein, g/L *Общий белок, г/л*	72.00 [69.00; 75.25]	72.00 [69.30; 74.25]	72.05 [71.00; 75.38]	0.953
Glucose, mmol/L *Глюкоза, ммоль/л*	5.34 [5.00; 6.18]	5.56 [4.90; 6.90]	4.75 [4.35; 5.48]	0.016
Serum iron, μmol/L *Железо, мкмоль/л*	19.00 [15.55; 24.16]	17.35 [13.75; 19.85]	12.35 [10.07; 18.50]	0.050
Serum HDL, mmol/L *ЛПВП, ммоль/л*	1.06 [0.90; 1.31]	1.12 [0.91; 1.33]	1.33 [1.23; 1.47]	0.016
Serum LDL, mmol/L *ЛПНП, ммоль/л*	3.19 [2.65; 4.06]	3.54 [2.93; 4.34]	3.10 [2.88; 3.45]	0.086
Serum VLDL, mmol/L *ЛПОНП, ммоль/л*	0.57 [0.44; 0.67]	0.78 [0.66; 1.16]	0.60 [0.39; 0.62]	< 0.0001
Serum cholesterol, mmol/L *Холестерин, ммоль/л*	4.69 [3.88; 5.77]	5.44 [4.66; 6.23]	4.82 [4.22; 5.47]	0.049
Potassium, mmol/L *Калий, ммоль/л*	4.40 [4.20; 4.60]	4.60 [4.30; 4.90]	4.35 [4.15; 4.50]	0.049
Creatinine, μmol/L *Креатинин, мкмоль/л*	76.00 [65.00; 93.75]	84.50 [76.00; 92.48]	81.50 [74.78; 89.25]	0.194
INR / МНО	1.27 [1.09; 1.55]	1.03 [0.96; 1.10]	1.03 [0.99; 1.08]	< 0.0001
Associated diseases ***Сопутствующие заболевания***				
Type 2 diabetes mellitus, *n* (%) *Сахарный диабет 2-го типа, n (%)*	5 (12.50 %)	17 (39.53 %)	(00.00 %)	0.0012
Hypertension, *n* (%) *Гипертоническая болезнь, n (%)*	11 (27.50 %)	29 (65.91 %)	(00.00 %)	< 0.0001
BMI, kg/m^2 *ИМТ, кг/м^2*	26.28 [22.20; 32.20]	30.60 [26.77; 33.60]	22.31 [20.90; 23.10]	< 0.0001
Variant of liver damage ***Вариант повреждения печени***				
Steatosis, *n* (%) *Стеатоз печени, n (%)*	2 (5.00 %)	22 (50.00 %)	(00.00 %)	< 0.0001
Steatohepatitis, *n* (%) *Стеатогепатит, n (%)*	7 (17.50 %)	14 (31.82 %)	(00.00 %)	
Cirrhosis, *n* (%) *Цирроз печени, n (%)*	31 (77.50 %)	8 (18.18 %)	(00.00 %)	

Note: *ALD — alcoholic liver disease; NAFLD — non-alcoholic fatty liver disease; HDL — high-density lipoproteins; LDL — low-density lipoproteins; VLDL — very low-density lipoproteins; INR — international normalized ratio, BMI — body mass index.*
Примечание: *АБП — алкогольная болезнь печени; НАЖБП — неалкогольная жировая болезнь печени; ЛПВП — липопротеины высокой плотности; ЛПНП — липопротеины низкой плотности; ЛПОНП — липопротеины очень низкой плотности; МНО — международное нормализованное отношение, ИМТ — индекс массы тела.*

Table 2. Principal components identified as a result of multifactorial analysis of metabolites in the overall cohort of study participants (the metabolites are distributed vertically in descending order of weight)

Таблица 2. Главные компоненты, выделенные в результаты многофакторного анализа метаболитов, в исследуемой популяции в общей когорте включенных в исследование людей (метаболиты распределены в порядке убывания весового коэффициента по вертикали)

MF I ***I МФ***	**MF II** ***II МФ***	**MF III** ***III МФ***	**MF IV** ***IV МФ***	**MF V** ***V МФ***	**MF VI** ***VI МФ***
O-palmitoyl-carnitine *Оксо-пальмитоил-карнитин* (C16-1)	Serine *Серин*	Lauroyl-carnitine *Лауроил-карнитин* (C12)	Valeryl-carnitine *Валерил-карнитин* (C5)	Pantothenic acid *Пантотеновая кислота*	Tyrosin *Тирозин*
Acetyl-carnitine *Ацетилкарнитин* (C2)	Choline *Холин*	Dodecenoyl-carnitine *Додеценоил-карнитин* (C12-1)	Propionyl-carnitine *Пропионил-карнитин* (C3)	Cortisol *Кортизол*	Methionine *Метионин*
Oleoyl-carnitine *Олеоилкарнитин* (C18-1)	Glycine *Глицин*	Tetradecenoyl-carnitine *Тетрадеценоил-карнитин* (C14-1)	Valine *Валин*	Xanthurenic acid *Ксантуреновая кислота*	Epinephrine *Эпинефрин*
Uridine *Уридин*	Dimethylglycine *Диметил-глицин*	Decanoyl-carnitine *Деканоил-карнитин* (C10)	Tiglyl-carnitine *Тиглил-карнитин* (C5-1)	Stearoyl-carnitine *Стеароил-карнитин* (C18)	
Linoleyl-carnitine *Линолеил-карнитин* (C18-2)	Phenylalanine *Фенилаланин*	Caproyl-carnitine *Капроил-карнитин* (C6)	Leucine *Лейцин*		
Metanephrine *Метанефрин*	Taurine *Таурин*	Myristoyl-carnitine *Миристоил-карнитин* (C14)	Carnitine *Карнитин* (C0)		
Asymmetric dimethylarginine *Асимметричный диметиларгинин*	Aspartic acid *Аспарагиновая кислота*		Lysine *Лизин*		
Total dimethylarginine *Общий диметиларгинин*			Butyryl-carnitine *Бутирил-карнитин* (C4)		
			Alanine *Аланин*		

Note: *MF — metabolomic factor; Metabolomic factor I (MF I) is represented by acetyl-carnitines: short-chain acetyl-carnitine, long-chain oleoyl-carnitine and linoleoyl-carnitine; nucleotide (uridine); metanephrine and two fractions of dimethylarginine — total and asymmetric; Metabolomic factor II (MF II) is represented by choline, a precursor of acetylcholine, and various amino acids: serine, glycine, aspartic acid, phenylalanine and their derivatives (dimethylglycine and taurine); Metabolomic factor III (MF III) consists of representatives of acetyl-carnitines: lauroyl-carnitine (C12), dodecanoyl-carnitine (C12-1), tetradecenoyl-carnitine (C14-1), decanoyl-carnitine (C10), caproyl-carnitine (C6) and myristoyl-carnitine (C14); Metabolomic factor IV (MF IV) is represented by acetyl-carnitines: valeryl-carnitine (C5), propionyl-carnitine (C3), tiglyl-carnitine (C5-1), carnitine (C0), butyryl-carnitine (C4); essential amino acids: valine, leucine, lysine and one replaceable amino acid — alanine; Metabolomic factor V (MF V) is represented by vitamin B5 (pantothenic acid), cortisol, tryptophan metabolite (xanthurenic acid) and stearoyl-carnitine (C18); Metabolomic factor VI (MF VI) is represented by metabolites of catecholamine metabolism: conditionally replaceable amino acid (tyrosine), epinephrine and metanephrine.*

Примечание: *МФ — метаболомный фактор; I метаболомный фактор (I МФ) представлен ацетилкарнитинами: короткоцепочечным ацетил-карнитином, длинноцепочечными олеоил-карнитином и линолеил-карнитином; нуклеотидом (уридином); метанефрином и двумя фракциями диметиларгенина — общим и асимметричным; II метаболомный фактор (II МФ) представлен холином, предшественником ацетилхолина, и различными аминокислотами: серином, глицином, аспарагиновой кислотой, фенилаланином и их производными (диметилглицином и таурином); III метаболомный фактор (III МФ) составляют представители ацетил-карнитинов: лауроил-карнитин (C12), додеценоил-карнитин (C12-1), тетрадеценоил-карнитин (C14-1), деканоил-карнитин (C10), капроил-карнитин (C6) и миристоил-карнитин (C14); IV метаболомный фактор (IV МФ) представлен ацетил-карнитинами: валерил-карнитином (C5), пропионил-карнитином (C3), тиглил-карнитином (C5-1), карнитином (C0), бутирил-карнитином (C4); незаменимыми аминокислотами: валином, лейцином, лизином и одной заменимой аминокислотой — аланином; V метаболомный фактор (V МФ) представлен витамином B5 (пантотеновой кислотой), кортизолом, метаболитом триптофана (ксантуреновой кислотой) и стеароил-карнитином (C18); VI метаболомный фактор (VI МФ) представлен метаболитами обмена катехоламинов: условно-заменимой аминокислотой (тирозином), эпинефрином и метанефрином.*

Table 3. Correlation matrix of metabolomic factors with clinical and laboratory data in patients
Таблица 3. Корреляционная матрица метаболомных факторов пациентов с клинико-лабораторными данными

Parameter / *Показатель*	I	II	III	IV	V	VI
BMI, kg/m^2 *ИМТ, кг/м^2*	0.04	0.15	0.03	0.31**	–0.01	0.25*
FIB-4 Index *Индекс FIB-4*	0.32**	0.01	0.12	–0.22*	0.19	0.61**
Laboratory parameters *Лабораторные показатели*						
Platelets, ×10^9/L *Тромбоциты, ×10^9/л*	–0.29**	0.04	0.00	0.13	–0.17	–0.63**
White blood cells,×10^9/L *Лейкоциты, ×10^9/л*	–0.15	0.14	0.02	0.24*	–0.06	–0.17
Red blood cells, ×10^{12}/L *Эритроциты, ×10^{12}/л*	–0.25*	0.14	–0.03	0.46**	–0.22*	–0.31**
Immunoglobulin A, g/L *Иммуноглобулин А, г/л*	0.31**	–0.04	0.21*	–0.45**	0.21*	0.39**
Immunoglobulin M, g/L *Иммуноглобулин М, г/л*	0.13	0.02	0.10	–0.13	0.22*	0.33**
Immunoglobulin G, g/L *Иммуноглобулин G, г/л*	0.16	0.00	0.19	–0.39**	0.05	0.44**
Serum total bilirubin, μmol/L *Билирубин общий, мкмоль/л*	0.33**	–0.14	0.18	–0.24*	0.07	0.49**
Serum direct bilirubin, μmol/L *Билирубин прямой, мкмоль/л*	0.27**	–0.12	0.23*	–0.33**	0.07	0.39**
Alanine aminotransferase, U/L *Аланинаминотрансфераза, Ед./л*	–0.16	0.02	0.01	0.15	0.10	0.11
Aspartate aminotransferase, U/L *Аспартатаминотрансфераза, Ед./л*	0.02	–0.02	0.14	–0.19	0.22*	0.54**
Gamma-glutamyl transferase, U/L *Гамма-глутамилтрансфераза, Ед./л*	0.19	0.14	0.19	–0.25*	0.39**	0.34**
Alkaline phosphatase, U/L *Щелочная фосфатаза, Ед./л*	0.18	–0.04	0.24*	–0.34**	–0.01	0.21*
Serum albumin, g/L *Альбумин, г/л*	–0.31**	0.05	0.01	0.37**	–0.08	–0.45**
Serum total protein, g/L *Общий белок, г/л*	–0.10	0.12	0.13	0.10	–0.04	–0.08
Glucose, mmol/L *Глюкоза, ммоль/л*	0.16	0.13	0.17	0.11	0.25*	0.07
Serum iron, μmol/L *Железо, мкмоль/л*	–0.01	0.10	0.10	0.12	–0.01	0.25*
Serum HDL, mmol/L *ЛПВП, ммоль/л*	–0.22*	–0.03	0.11	–0.10	–0.20	–0.18
Serum LDL, mmol/L *ЛПНП, ммоль/л*	–0.09	0.15	–0.08	0.14	0.15	–0.18
Serum VLDL, mmol/L *ЛПОНП, ммоль/л*	0.02	0.24*	–0.11	0.36**	0.15	–0.09
Serum cholesterol, mmol/L *Холестерин, ммоль/л*	–0.15	0.15	–0.05	0.14	0.1	–0.2*
Potassium, mmol/L *Калий, ммоль/л*	0.1	0.03	–0.16	0.04	0.03	–0.28**
Creatinine, μmol/L *Креатинин, мкмоль/л*	–0.06	–0.07	–0.09	0.36**	–0.16	–0.16
INR / *МНО*	0.24*	–0.10	0.00	–0.39**	0.16	0.48**

Note: *BMI — body mass index; HDL — high-density lipoproteins; LDL — low-density lipoproteins; VLDL — very low-density lipoproteins; INR — international normalized ratio; * — statistical significance < 0.05; ** — statistical significance < 0.001.*

Примечание: *ИМТ — индекс массы тела; ЛПВП — липопротеины высокой плотности; ЛПНП — липопротеины низкой плотности; ЛПОНП — липопротеины очень низкой плотности; МНО — международное нормализованное отношение; * — статистическая значимость < 0,05; ** — статистическая значимость < 0,001.*

The patient groups showed statistically significant differences in immunoglobulin fractions. IgA and IgG were elevated in patients with ALD compared to those with NAFLD ($p = 0.0004$) and the control group ($p = 0.015$). Compared to ALD, a decrease in IgM concentration was observed in NAFLD ($p < 0.0001$).

The ALD group differed significantly from the NAFLD ($p < 0.0001$) and control groups ($p = 0.015$) by the presence of pronounced coagulopathy, with an increased international normalised ratio (INR), likely due to a higher proportion of patients with liver cirrhosis (LC) in the ALD group.

In the course of our study, the levels of 96 metabolites of various biochemical pathways were analyzed. To better assess the interrelations between the studied metabolites in the presented sample, and their influence on disease development and progression, we performed a multifactorial analysis. As a result, six principal components were identified, with a cumulative explained variance of 70.0 %. Each metabolomic factor (MF) is represented by metabolites with the highest factor loadings (greater than 0.53) and presented in Table 2 in descending order of weighting coefficient.

To assess the relationship between metabolites and disturbances in systemic homeostasis in the context of disease development and progression, we conducted a correlation analysis of the principal factors with clinical and laboratory parameters of the patients. The results of the correlation analysis are presented in Table 3.

The analysis results allowed us to identify associations between patients' metabolomic profiles and laboratory findings. In interpreting the data obtained, statistically significant correlations were noted. Thus, the relationship between the factors and liver cirrhosis development is reflected by a statistically significant positive correlation between the FIB-4 index and metabolites within MF I ($p < 0.001$) and MF VI ($p < 0.001$), and a negative correlation with MF IV ($p < 0.05$).

An increase in metabolites of MF I showed statistically significant correlations with thrombocytopenia ($p < 0.001$), anemia ($p < 0.05$), as well as hypoalbuminemia ($p < 0.001$) and decreased HDL ($p < 0.05$). Metabolomic factor I was also associated with hyperbilirubinemia ($p < 0.001$), hypocoagulation ($p < 0.05$), and elevated IgA levels ($p < 0.001$).

Metabolomic factor II positively correlated with VLDL levels ($p < 0.05$).

A positive correlation was observed between metabolomic factor III and ALP, direct bilirubin, and IgA levels ($p < 0.05$).

Metabolites of MF IV were mostly associated with reduced levels of cholestasis markers (alkaline phosphatase, gamma-glutamyl transferase), bilirubin, INR, and immunoglobulin fractions (IgG and IgA). At the same time, a positive correlation was observed between metabolites concentrations in serum and albumin levels, as well as increased leukocyte and erythrocyte counts in the complete blood count.

Metabolomic factor V was associated with increased secretion of immunoglobulin fractions A and M. A positive correlation was noted with hyperglycemia, elevated gamma-glutamyl transferase and aspartate aminotransferase levels, and a tendency toward anemia in the complete blood count.

The strongest correlations were observed with metabolomic factor VI. Alongside a significant positive correlation between metabolite levels and the calculated FIB-4 index, there was an interconnection with thrombocytopenia in the complete blood count. In patients, a predominance of MF VI correlated with hyperbilirubinemia, hypoalbuminemia, elevated cholestasis markers (alkaline phosphatase and gamma-glutamyl transferase), alanine aminotransferase levels, and imbalanced secretion of all immunoglobulin fractions.

We also conducted a comparative analysis of differences in metabolomic factors across patients with different disease etiologies and, in comparison, with the control group (Table 4).

MF I showed statistically significant differences between both ALD patients ($p = 0.003$) and NAFLD patients ($p = 0.015$) compared to the control group. In patients with liver disease, unlike healthy volunteers, there was an increase in metabolic pathway activity and elevated levels of all metabolites (Fig. 1).

Metabolomic factors IV and VI significantly differentiated ALD patients from NAFLD patients ($p = 0.012$ and $p = 0.011$, respectively). In the context of toxic etiology of liver disease, an increase in the activity of MF VI and a decrease in the activity and concentration of metabolites within metabolomic factor IV were observed.

Given the presence of correlations between metabolomic factors and clinical-laboratory parameters characteristic of liver cirrhosis, a comparative analysis was conducted between patients with cirrhosis and the general cohort of individuals without cirrhosis, including those from the control group. The inclusion of healthy volunteers in the factor analysis is justified from the perspective of enhancing the reliability and interpretability of the identified latent variables. This contributes to proper model calibration,

Table 4. Differences in the metabolomic profile of patients with alcoholic liver disease, non-alcoholic fatty liver disease, and the control group

Таблица 4. Различия метаболомного профиля пациентов с алкогольной болезнью печени, пациентов с неалкогольной жировой болезнью печени и группы контроля

MF *МФ*	ALD *АБП* (n = 40)	NAFLD *НАЖБП* (n = 44)	Control group *Группа контроля* (n = 14)	p	ALD / NAFLD *АБП / НАЖБП*	ALD / Control group *АБП / Группа контроля*	NAFLD / Control group *НАЖБП / Группа контроля*
	Me [Q_1; Q_3]						
I	0.02 [–0.41; 0.40]	–0.14 [–0.29; 0.13]	–0.47 [–0.53; –0.35]	0.003	0.767	0.003	0.015
II	–0.31 [–0.83; 0.19]	–0.09 [–0.42; 0.74]	–0.24 [–0.59; –0.05]	0.137	0.186	0.998	0.398
III	–0.14 [–0.55; 0.28]	–0.16 [–0.51; 0.35]	–0.09 [–0.46; 0.26]	0.981	0.981	0.997	0.997
IV	–0.37 [–1.11; 0.35]	0.29 [–0.30; 0.84]	–0.05 [–0.54; 0.49]	0.012	0.012	0.545	0.609
V	–0.08 [–0.29; 0.13]	–0.10 [–0.22; 0.01]	–0.22 [–0.37; –0.15]	0.061	0.983	0.078	0.098
VI	0.24 [–0.38; 1.15]	–0.34 [–0.63; 0.11]	–0.54 [–0.70; –0.41]	< 0.0001	0.011	0.0002	0.136

Note: *MF — metabolomic factor; ALD — alcoholic liver disease; NAFLD — non-alcoholic fatty liver disease.*
Примечание: *МФ — метаболомный фактор; АБП — алкогольная болезнь печени; НАЖБП — неалкогольная жировая болезнь печени.*

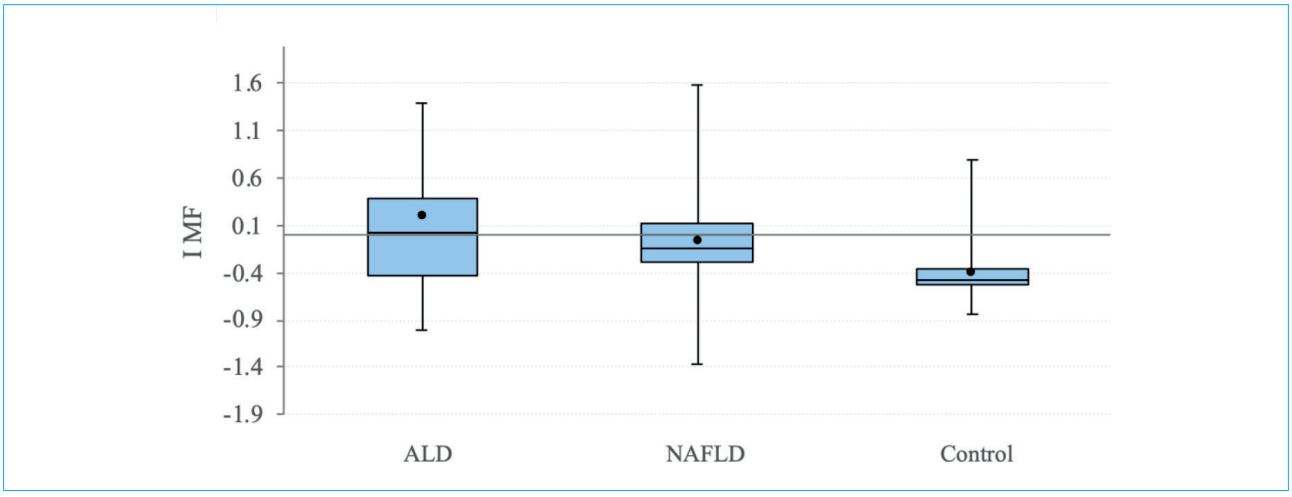

Figure 1. Differences in the metabolomic profile I against the background of diseases of various etiologies in comparison with the control group

Рисунок 1. Различие метаболомного профиля I на фоне заболеваний различной этиологии в сравнении с группой контроля

helps to distinguish natural data variability from pathological variations, and provides an objective assessment of the contribution of each identified factor to the total variance. Furthermore, the combined sample increases the statistical power of the analysis, which is particularly important when studying complex biochemical systems where patterns may be subtle in relatively homogeneous patient groups with limited variability.

We identified statistically significant, oppositely directed changes in the metabolomic profiles of patients with a confirmed diagnosis of liver cirrhosis versus those without cirrhosis and the results are presented in Table 5.

In patients with liver cirrhosis of various etiologies (ALD and NAFLD), an increased activity of metabolic pathways associated with MF I (p = 0.003), MF V (p = 0.002), and MF VI

Table 5. Differences in the metabolomic profile of patients with and without liver cirrhosis
Таблица 5. Различия метаболомного профиля пациентов с циррозом печени и без цирроза печени

Metabolomic factor ***Метаболомный фактор***	**Patients with liver cirrhosis** ***Пациенты с циррозом печени*** **(*n* = 39)**	**Patients without liver cirrhosis** ***Пациенты без цирроза печени*** **(*n* = 59)**	***p***
	***Me* [Q_1; Q_3]**		
I	0.13 [–0.20; 0.42]	–0.23 [–0.43; –0.01]	0.003
II	–0.07 [–0.47; 0.51]	–0.26 [–0.63; 0.17]	0.351
III	–0.06 [–0.55; 0.35]	–0.16 [–0.48; 0.32]	0.588
IV	–0.47 [–1.02; 0.24]	0.31 [–0.31; 0.81]	0.0002
V	–0.02 [–0.15; 0.14]	–0.19 [–0.30; –0.05]	0.002
VI	0.50 [–0.15; 1.22]	–0.42 [–0.67; –0.19]	< 0.0001

Table 6. Differences in the metabolomic profile of patients with liver cirrhosis resulting from alcoholic liver disease and non-alcoholic fatty liver disease
Таблица 6. Различия метаболомного профиля пациентов с циррозом печени в исходе алкогольной болезни печени и неалкогольной жировой болезни печени

Metabolomic factor ***Метаболомный фактор***	**Liver cirrhosis as a result of ALD** ***Цирроз печени в исходе АБП*** **(*n* = 31)**	**Liver cirrhosis as a result of NAFLD** ***Цирроз печени в исходе НАЖБП*** **(*n* = 8)**	***p***
	***Me* [Q_1; Q_3]**		
I	0.14 [–0.12; 0.45]	0.12 [–0.36; 0.16]	0.531
II	–0.16 [–0.72; 0.23]	0.54 [0.05; 1.27]	0.007
III	–0.12 [–0.46; 0.21]	0.38 [–0.55; 0.52]	0.347
IV	–0.66 [–1.15; 0.24]	–0.23 [–0.59; 0.20]	0.347
V	–0.03 [–0.18; 0.14]	–0.02 [–0.11; 0.10]	0.531
VI	0.72 [–0.15; 1.46]	0.18 [–0.11; 0.46]	0.125

Note: *ALD — alcoholic liver disease; NAFLD — non-alcoholic fatty liver disease.*
Примечание: *АБП — алкогольная болезнь печени; НАЖБП — неалкогольная жировая болезнь печени.*

($p < 0.001$) was observed, with a trend toward elevated concentrations of metabolites in serum. In contrast, for MF IV ($p < 0.001$), a statistically significant trend toward decreased metabolic activity and lower metabolite levels was revealed.

To assess the influence of etiological factors on metabolome changes in patients with liver cirrhosis, a subgroup analysis was conducted among patients with cirrhosis resulting from ALD and NAFLD.

In the analysis of metabolomic profiles in cirrhosis subgroups, statistically significant differences were identified in relation to MF II among the patients with cirrhosis of NAFLD origin — an increase in serum metabolite levels was observed compared to the patients with cirrhosis of ALD origin ($p = 0.007$) (Table 6).

To assess the relationship between metabolomic factors and disease severity, we conducted a comparative analysis of patients with liver cirrhosis at different stages of the disease according to the Child — Pugh classification, compared with patients without liver cirrhosis (Table 7).

In the analysis of patients with liver cirrhosis, statistically significant differences were noted across cirrhosis severity classes according to the Child — Pugh scale. A trend toward a decrease in metabolite levels within metabolomic profile II was observed with disease progression. Child — Pugh class A cirrhosis differed statistically significantly from class C ($p = 0.0047$) (Fig. 2).

As the disease progressed, a trend toward increased metabolic activity within metabolomic profile VI was observed. The metabolite levels differed significantly between patients without cirrhosis and those with class B and C cirrhosis according to the Child — Pugh scale ($p < 0.0001$) (Fig. 3).

We have noted a tendency towards multi-directional changes in metabolite concentrations as the disease progressed: an increase in the levels of metabolites in MF I and a decrease in the activity of metabolic pathways represented by MF IV. However, no statistically significant differences were found within the patient subgroups.

MF III, which primarily reflects the metabolism of long-chain acylcarnitines, did not show differences depending on the disease etiology or cirrhosis status, which may be explained by the relatively small sample size.

Table 7. Differences in the metabolomic profile of patients with liver cirrhosis at various stages of severity according to the Child — Pugh classification, compared with patients without liver cirrhosis

Таблица 7. Различия метаболомного профиля пациентов с циррозом печени на разных стадиях тяжести заболевания по классификации Чайлда — Пью в сравнении с пациентами без цирроза печени

Metabolomic factor ***Метаболомный фактор***	**Patients without liver cirrhosis** ***Пациенты без цирроза печени*** **(*n* = 59)**	**Patients with liver cirrhosis (Child — Pugh classification)** ***Пациенты с циррозом печени (по классификации Чайлда — Пью)***			***p***
		Class A ***Класс А*** **(*n* = 16)**	**Class B** ***Класс В*** **(*n* = 15)**	**Class C** ***Класс С*** **(*n* = 8)**	
I	–0.23 [–0.43; –0.01]	–0.05 [–0.69; 0.16]	0.19 [–0.02; 0.44]	0.30 [0.01; 0.73]	0.0059
II	–0.26 [–0.63; 0.17]	0.46* [–0.06; 0.93]	–0.14 [–0.51; 0.17]	–0.70* [–0.95; –0.36]	0.0023
III	–0.16 [–0.48; 0.32]	–0.12 [–0.55; 0.35]	–0.12 [–0.36; 0.16]	0.10 [–0.29; 0.59]	0.8017
IV	0.31 [–0.31; 0.81]	–0.23 [–0.66; 0.32]	–0.79 [–0.96; 0.03]	–0.99 [–1.70; –0.16]	0.0012
V	–0.19 [–0.30; –0.05]	–0.08 [–0.13; 0.10]	0.07 [–0.06; 0.17]	–0.11 [–0.21; –0.01]	0.0108
VI	–0.42* [–0.67; –0.19]	–0.11 [–0.42; 0.46]	0.76* [0.52; 1.71]	0.76* [0.29; 1.43]	<0.0001

Note: ** — differences are statistically significant ($p < 0.05$).*
Примечание: ** — различия статистически значимы ($p < 0,05$).*

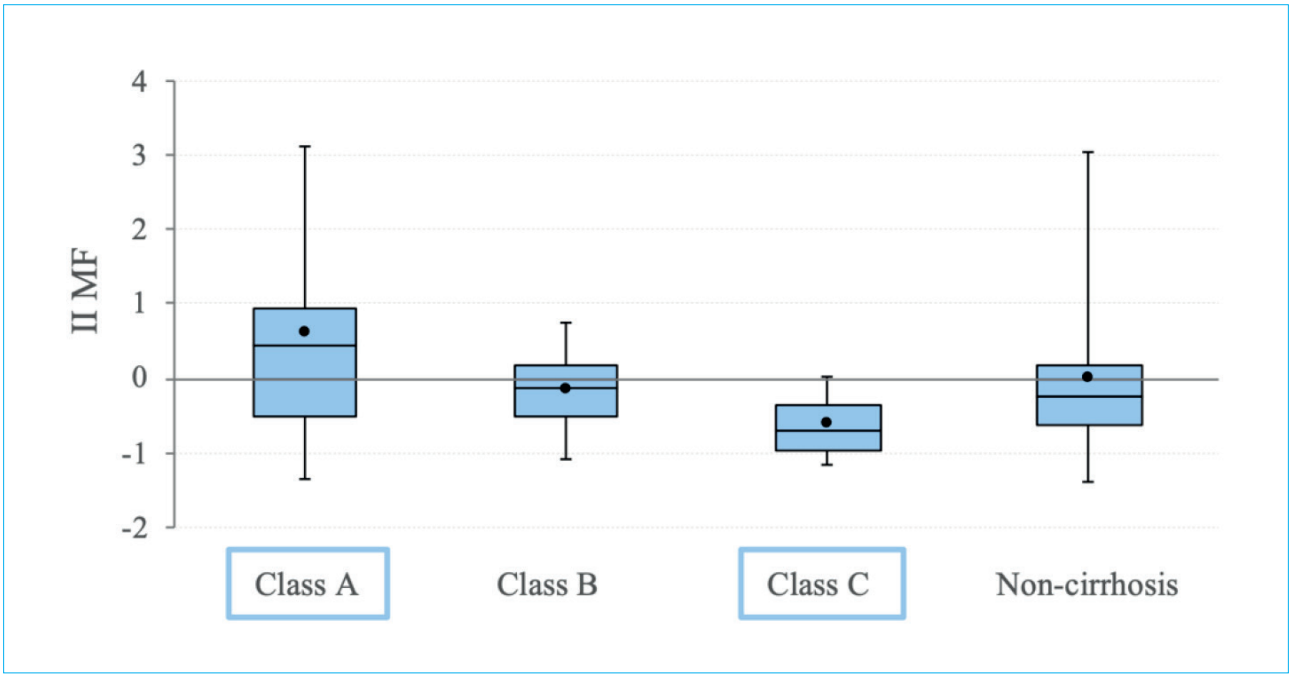

Figure 2. Differences in metabolomic profile II during disease progression compared with the group of patients without liver cirrhosis

Рисунок 2. Различие метаболомного профиля II на фоне прогрессирования заболевания в сравнении с группой пациентов без цирроза печени

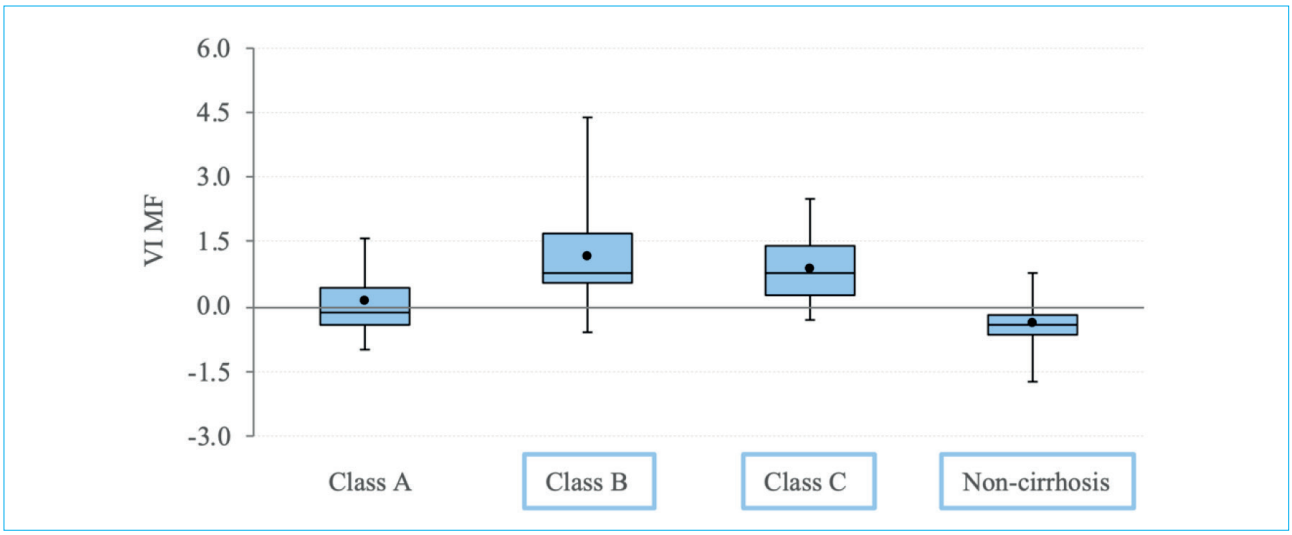

Figure 3. Differences in metabolomic profile VI during disease progression compared with the group of patients without liver cirrhosis
Рисунок 3. Различие метаболомного профиля VI на фоне прогрессирования заболевания в сравнении с группой пациентов без цирроза печени

Discussion

The metabolomic profile reflects all biochemical processes continuously occurring within the human body, both at the intracellular and extracellular levels. During disease development, the activation of numerous latent compensatory mechanisms is observed, and the subsequent failure of the organism's adaptation to pathological internal and external influences leads to decompensation and disease progression. From a clinical standpoint, research in the field of metabolomics is extremely important both for the search for early biomarkers of disease at the preclinical stage and for the development of therapies aimed at regulating specific biochemical pathways.

Changes in the metabolomic profile in the context of various diseases have recently become an active area of investigation. One of the main challenges in this field is the high cost of metabolomic studies. Most of international studies on liver disease-related metabolomics evaluate the levels of individual groups of metabolites in biological fluids and tissues.

In our study, we performed a one-time targeted metabolomic analysis of 96 serum metabolites in patients. A large set of analyzed molecules allows for a more comprehensive characterization of metabolomic profile changes in the studied disease forms. In the course of the analysis, we identified six principal components, for which we propose the term "metabolomic factor". These metabolomic factors, consisting of various bioactive molecules, may reflect both compensatory and decompensatory processes in the body during disease, as well as serve as damaging contributors, driving disease progression. Upon more detailed analysis of patient groups and the control group, statistically significant differences in the metabolic activity of individual metabolomic factors were identified, which collectively more accurately characterize changes in the metabolomic profile of our patients. Based on the data obtained, it is possible in future studies to explore the biochemical interactions of specific metabolomic factors and their role in disease development.

The data we obtained largely agree with previously published findings.

Many studies have shown that the development and progression of NAFLD are based on metabolic disturbances that indirectly lead to mitochondrial dysfunction, oxidative stress, hepatocyte injury, immune system activation, and the development of fibrosis [5]. A specific role in the pathogenesis of NAFLD is attributed to amino acid metabolism. Among the essential amino acids, there are three branched-chains amino acids (BCAAs) — leucine, valine, and isoleucine. BCAA catabolism involves the enzyme branched-chain amino acid aminotransferase (BCAT), which forms the corresponding α-keto acids, with subsequent involvement of the metabolites in adenosine triphosphate (ATP) production and entry into the Krebs cycle [6]. However, an alternative biochemical pathway is possible, in which active BCAA metabolites participate in lipogenesis. This occurs through the conjugation of carnitine with BCAA catabolism products and their transport

into the cytoplasm via a specific transporter. Studies involving obese patients have reported an association between BCAAs and acylcarnitines C3 and C5 [7]. P. Sánchez-Pintos et al. identified elevated C3 and C5 levels in the blood of newborns who were large for their gestational age and at increased risk of developing insulin resistance, obesity, and metabolic syndrome later in life [8]. In their work, T. Kazuto et al. proposed a hidden biochemical link between L-carnitine (C0) and BCAA and its role in the development of hepatic encephalopathy in LC [9]. Studies investigating the effects of BCAA on liver metabolism have shown that catabolism of these molecules is reduced in NAFLD, leading to elevated levels of amino acids in the serum of affected patients [10, 11]. It is also known that BCAAs, as nitrogen donors, participate in the synthesis of alanine from pyruvate in the Krebs cycle [12]. Therefore, under altered BCAA metabolism, an increase in alanine levels is presumed. These findings are consistent with our results, in which we observed elevated concentrations of metabolites in MF IV, consisting of short-chain acyl-carnitines and amino acids, in patients with NAFLD.

In our study, an association was identified between metabolomic factors I, V, and VI and the presence of liver cirrhosis in the cohort of patients included in the study. A positive correlation was established between MF I and VI and the FIB-4 index. We have analyzed the metabolomic profile of patients depending on disease severity according to the Child — Pugh scale, taking into account the presence of ascites, hepatic encephalopathy, hypocoagulation, bilirubin level, and decreased protein-synthetic function of the liver. As the disease progressed, a significant increase in the metabolic activity of MF VI was observed, suggesting that the levels of methionine, tyrosine, and epinephrine may reflect the progression of liver failure, jaundice, and impaired liver detoxification function. These clinical manifestations are supported by the established correlation between MF VI and changes in biochemical blood analysis in the studied cohort.

Methionine is an essential proteogenic amino acid [13]. Recent studies have shown that disturbances in methionine metabolism and functional alterations of related enzymes are closely associated with fibrosis progression and the development of hepatocellular carcinoma [14]. The role of the active catabolite of methionine, S-adenosylmethionine (SAM), in maintaining homeostasis has been well described. Evidence suggests that SAM can exert a direct antioxidant effect, prevent mitochondrial DNA damage and mitoribosome dissociation, and suppress pro-inflammatory cytokines [15]. In addition, SAM is the main methyl group donor for the synthesis of phosphatidylcholine, which is essential for the export of VLDL from the liver [16]. It has been demonstrated that patients with insulin resistance and non-alcoholic steatohepatitis show a reduced rate of methionine transmethylation compared to the control group [17].

According to many observations, the liver is one of the primary organs involved in the metabolism of aromatic amino acids (AAAs: phenylalanine, tyrosine, and tryptophan) [18]. Literature data describe changes observed in patients with liver cirrhosis, including elevated levels of AAAs, in particular phenylalanine and tyrosine, and reduced levels of BCAAs in plasma [19]. In their work, G. Dam et al. showed that the BCAA/AAA ratio, the so-called "Fisher ratio", negatively correlated with disease severity and survival scores according to the Child — Pugh classification [20]. Many studies demonstrate that in the context of hyperammonemia, which may occur in liver cirrhosis, catabolism of branched-chain amino acids is enhanced, leading to a decrease in BCAA levels in plasma [21]. Similar changes in the metabolomic profile of patients with chronic liver disease were noted by M. Gaggini et al. [22]. These findings are consistent with the metabolomic alterations identified in our study. We found a statistically significant association of metabolomic factors IV and VI with the FIB-4 index. In the context of liver cirrhosis, we observed oppositely directed changes in metabolite concentrations, specifically, a decrease in BCAAs (valine and leucine) and an increase in AAA (tyrosine) in cirrhotic patients. However, phenylalanine was included in metabolomic factor II, and its concentration decreased as LC progressed.

Alterations in catecholamine metabolism during the development and progression of liver diseases have been described in the literature. In our study, statistically significant changes were found in the levels of epinephrine and its metabolite, metanephrine. However, during analysis, these molecules were grouped into different metabolomic factors. The researches indicate that the liver interacts directly with catecholamines via the sympathetic nervous system. It has been demonstrated that nerve endings contact hepatocytes, stellate cells, sinusoidal endothelial cells, as well as bile ducts [23]. It has been established that catecholamines in the liver are able to regulate glucogenolysis and gluconeogenesis [24, 25]. The studies in animal models have shown that catecholamines can directly regulate intracellular triglyceride breakdown into free fatty acids and restrict VLDL secretion [26, 27]. Both animal and human studies have shown that

plasma catecholamine levels increase with alcohol consumption and in patients with liver cirrhosis of various etiologies [28].

Along with metabolomic factors I and VI, we also identified statistically significant increases in vitamin B_5, cortisol, xanthurenic acid, and acylcarnitine (C18) in patients with liver cirrhosis compared to those without it. Previously, we demonstrated that the tryptophan metabolite xanthurenic acid was significantly elevated in patients with hepatic encephalopathy [29]. However, regarding cortisol, our results partially diverge from the literature. Several studies have shown that as liver cirrhosis progresses, cortisol levels may decrease as a manifestation of hepatoadrenal syndrome [30].

Another metabolite associated with liver diseases, and which function has been well described in the literature is asymmetric dimethylarginine (ADMA). It has been established that this metabolite, which is linked to the risk of cardiovascular complications, increases with the progression of liver failure [31]. In our study, an elevated level of this metabolite was observed within MF I in patients with liver cirrhosis.

In recent years, many studies have focused on mitochondrial dysfunction and its role in the pathogenesis of various diseases. According to the literature, mitochondrial dysfunction underlies metabolic syndrome, lipid metabolism disorders and insulin resistance [32]. Previously, we had demonstrated that in patients with liver cirrhosis resulting from ALD and NAFLD, there is a decrease in the levels of short-chain fatty acids (SCFAs) in stool samples [33]. SCFAs serve as substrates for mitochondrial function, and their reduction may potentially lead to mitochondrial dysfunction [34]. In this study, a large number of metabolites were identified that are directly or indirectly involved in energy metabolism. Specifically, we observed increased levels of long-chain acylcarnitines, which may result from impaired function of the carnitine fatty acid transporter and potentially reflect damage to the mitochondrial membrane [35]. We also noted elevated levels of vitamin B_5 in patients with liver cirrhosis. This metabolite is a key component of coenzyme A and is involved in the β-oxidation of fatty acids and the tricarboxylic acid cycle [36]. Thus, an elevated level of pantothenic acid, associated with disease progression, may also indicate disturbances in mitochondrial energy metabolism. An increased level of methionine in patients with LC is the result of impaired metabolism of this molecule to S-adenosylmethionine and thus is associated with enhanced mitochondrial damage in the context of oxidative stress [37].

According to metabolomic research, the liver is a central organ in which numerous biochemical processes take place. Currently, the literature describes alterations in the metabolism of specific classes of compounds in a number of disease conditions. In our study, we have presented the results of multivariate analysis that allow us to characterize the main changes in the metabolomic profiles of patients with NAFLD and ALD. The findings are consistent with previously described alterations in the human metabolome observed in the context of liver injury associated with ALD and NAFLD, as well as in liver cirrhosis [38]. In addition, the obtained data provide insight into hidden biochemical interactions within pathophysiological processes. We have identified correlation patterns between metabolomic factors and clinical-laboratory parameters. Variability in metabolomic profiles may be considered both a cause and a consequence of disease development and progression. To further clarify these findings, prospective studies involving larger patient cohorts are essential.

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