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Diagnostic Role of Various Short-Chain Fatty Acids in Blood of Patients with Chronic Heart Failure Complicated by Sarcopenia

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Aim: to investigate the levels of various short-chain fatty acids (SCFAs) in the blood of patients with chronic heart failure (CHF) complicated by sarcopenia and to analyze their associations with clinical parameters of CHF and sarcopenia.
Materials and methods. This study included 76 patients with CHF (mean age — 68.0 ± 9.8 years). Plasma levels of SCFAs (e.g., acetic, propionic, and butyric acids) were determined using electrospray ionization mass spectrometry (ESI-MS). Sarcopenia was diagnosed according to the EWGSOP2 algorithm, which included assessments of muscle strength (mechanical dynamometry), muscle mass (bioimpedance analysis), and muscle function (SPPB tests).
Results. The plasma concentrations of butyric acid (C4) were on average 15,050 ng/mL (95 % CI: 12,525–18,200) in patients without sarcopenia and 16,400 ng/mL (95 % CI: 10,100–20,850) in patients with sarcopenia ($p = 0.00003$). Pentanoic acid (C5) levels were significantly lower: 472.0 ng/mL (95 % CI: 368.2–551.2) in patients without sarcopenia versus 439.0 ng/mL (95 % CI: 348.0–514.5) in patients with sarcopenia ($p = 0.00001$). Four distinct clusters of CHF patients were identified based on their clinical, laboratory, and SCFA profiles. These clusters reflected differences in the severity of CHF, the presence of sarcopenia, physical activity levels, and SCFA concentrations. The most pronounced differences in SCFA levels were observed between Clusters 2 and 3 (C4 levels: 18,650 ng/mL (95 % CI: 14,950–21,950) and 10,600 ng/mL (95 % CI: 9,220–12,600), respectively).
Conclusions. Differences in SCFA levels between clusters indicate potential links between SCFA metabolism, CHF, and sarcopenia progression. These differences may serve as biomarkers for identifying patients at high risk for sarcopenia and highlight the need for an individualized treatment approach, including SCFA metabolism and gut microbiota modulation.
Keywords: chronic heart failure, sarcopenia, short-chain fatty acids
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Диагностическая роль уровня короткоцепочечных жирных кислот в крови у пациентов с хронической сердечной недостаточностью, осложненной саркопенией

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Цель исследования: изучить уровни различных короткоцепочечных жирных кислот (КЦЖК) в крови у пациентов с хронической сердечной недостаточностью (ХСН), осложненной саркопенией, и проанализировать их взаимосвязь с клиническими параметрами ХСН и саркопении.

Материалы и методы. В исследование включены 76 пациентов с ХСН (средний возраст — 68,0 ± 9,8 года). Уровни КЦЖК (уксусная, пропионовая, бутановая и др.) в плазме крови определялись методом масс-спектрометрии с ионизацией распылением в электрическом поле (ESI). Диагностика саркопении проводилась согласно алгоритму EWGSOP2 и включала измерение мышечной силы (механический динамометр), мышечной массы (биоимпедансометрия), мышечной функции (тесты SPPB).
Результаты. Концентрации бутановой кислоты (С4) в плазме крови составили в среднем 15 050 нг/мл (95%-ный доверительный интервал (95% ДИ): 12 525–18 200) у пациентов без саркопении и 16 400 нг/мл (95% ДИ: 10 100–20 850) у пациентов с саркопенией (p = 0,00003). Уровни пентановой кислоты (С5) оказались значительно ниже: 472,0 нг/мл (95% ДИ: 368,2–551,2) у пациентов без саркопении и 439,0 нг/мл (95% ДИ: 348,0–514,5) у пациентов с саркопенией (p = 0,00001). В исследовании были выделены четыре кластера пациентов с ХСН на основе их клинико-лабораторных показателей и уровней КЦЖК. Кластеры отражают различия в тяжести сердечной недостаточности, наличии саркопении, уровне физической активности среди пациентов и различия по уровням КЦЖК. Наиболее выраженные различия по уровням КЦЖК были выявлены между кластерами 2 и 3 (уровни С4: 18 650 нг/мл (95% ДИ: 14 950–21 950) и 10 600 нг/мл (95% ДИ: 9 220–12 600) соответственно).
Выводы. Различия в уровнях КЦЖК между кластерами указывают на возможные связи между метаболизмом КЦЖК, сердечной недостаточностью и прогрессированием саркопении. Эти различия могут служить биомаркерами для выявления пациентов с высоким риском саркопении и предполагают необходимость индивидуализированного подхода в лечении, включая коррекцию метаболизма КЦЖК и микробиоты кишечника.
Ключевые слова: хроническая сердечная недостаточность, саркопения, короткоцепочечные жирные кислоты
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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Introduction

The presence of chronic heart failure (CHF) significantly increases the risk of sarcopenia. According to the SICA-HF study [1], the prevalence of sarcopenia in CHF patients was 20 % higher compared to age-matched individuals without CHF. Among patients aged 65 and older, CHF increased the risk of sarcopenia by 3.54 times (p = 0.013) [2]. It is well-established that the development of sarcopenia, in turn, worsens the course and prognosis of CHF [3]. In this context, the analysis of the effects of various biological substrates on muscle tissue function is of considerable clinical interest.

In recent years, the impact of gut microbiota on skeletal muscle function has gained attention. One of the mediators in this process is short-chain fatty acids (SCFAs), which are produced by gut microbiota [4]. Changes in gut microbiota composition, and consequently in SCFA levels, are associated with increased subclinical chronic inflammation [5], which triggers muscle tissue damage and the progression of sarcopenia [6, 7]. Thus, studying the levels and ratios of various SCFAs in patients with CHF and sarcopenia is of paramount importance.

Aim of the study

The objective of this study was to investigate the blood levels of various SCFAs in patients with CHF complicated by sarcopenia and to analyze their associations with clinical parameters of CHF and sarcopenia.

Materials and methods

The study was conducted at Moscow City Clinical Hospital No. 4 of the Moscow Department of Health from September 2019 to December 2021 and was approved by the hospital's Ethics Committee. A total of 76 patients meeting the inclusion/exclusion criteria (Table 1) were enrolled in the study.

Sarcopenia was diagnosed using the EWGSOP2 algorithm [8]. Muscle strength of skeletal muscles was assessed with a mechanical hand dynamometer DK-25 (Nizhny Tagil Medical Instrument Plant, Russia). Muscle mass was assessed using a bioimpedance analyzer of metabolic processes and body composition ABC-02 "Medass" (Russia) using the basic program for assessing integral parameters of body composition ABC02-0362. Muscle function was evaluated using tests comprising the Short Physical Performance Battery (SPPB) [9].

All patients underwent a comprehensive clinical examination, including echocardiography to assess left ventricular ejection fraction and blood sampling to determine NTproBNP (N-terminal prohormone of brain natriuretic peptide) levels and SCFA concentrations.

SCFA levels in plasma samples were measured at the Department of Medical Chemistry and Toxicology, Pirogov Russian National Research Medical University. Analysis was performed using electrospray ionization mass spectrometry (ESI-MS) in positive ionization mode. Multiple Reaction Monitoring (MRM) transitions were used for:

Table 1. Criteria for inclusion and exclusion in the study
Таблица 1. Критерии включения и невключения в исследование

Inclusion criteria ***Критерии включения***	1. Men and women over 18 years old / *Мужчины и женщины старше 18 лет*
	2. Verified diagnosis of CHF stage 2B / *Верифицированный диагноз ХСН стадии 2Б*
Non-inclusion criteria ***Критерии невключения***	1. Having a pacemaker / *Наличие электрокардиостимулятора*
	2. Any severe, decompensated or unstable somatic diseases or conditions that, in the opinion of the researcher, threaten the patient's life or worsen the prognosis of the disease (including anemia, autoimmune, endocrine, oncological diseases, hepatitis, connective tissue diseases, etc.) / *Любые тяжелые, декомпенсированные или нестабильные соматические заболевания или состояния, которые, по мнению исследователя, угрожают жизни больного или ухудшают прогноз заболевания (в том числе анемия, аутоиммунные, эндокринные, онкологические заболевания, гепатиты, заболевания соединительной ткани и др.)*
	3. Long-term bed rest / *Длительный постельный режим*
	4. Any severe congenital and acquired injuries of the musculoskeletal system / *Любые тяжелые врожденные и приобретенные повреждения опорно-двигательного аппарата*
	5. Concomitant neurosurgical or neurological pathology / *Наличие сопутствующей нейрохирургической или неврологической патологии*
	6. Abuse of alcohol, drugs, and narcotics / *Злоупотребление алкоголем, лекарственными средствами, наркотическими веществами*
	7. Mental illness, legal incapacity / *Психические заболевания, недееспособность*

Note: *CHR — chronic heart failure.*
Примечание: *ХСН — хроническая сердечная недостаточность.*

• propionic acid derivative (C3): 180.05 ng/mL → 91.05 ng/mL
• butyric (C4) and isobutyric acids (iC4): 194.1 ng/mL → 91.05 ng/mL
• pentanoic (C5), 3-methylbutanoic (βC5), and 2-methylbutanoic acids (αC5): 208.15 ng/mL → 91.05 ng/mL
• 4-methylpentanoic (iC6) and hexanoic acids (C6): 222.10 ng/mL → 91.05 ng/mL.

Concentrations of 3-methylpentanoic and 4-methylpentanoic acids were below the detection limit, while acetic acid levels exceeded the upper limit of the analytical range. Therefore, these acids were excluded from the study.

Machine learning

To determine the relationships between SCFAs levels and sarcopenia, machine learning techniques utilizing classification methods were employed, including Principal Component Analysis (PCA) and the k-means clustering algorithm. An unsupervised learning approach was applied, enabling the model to independently explore the data, identify patterns, and uncover correlations.

For effective implementation of machine learning methods, incomplete, duplicate, and inconsistent data that could negatively impact the results were removed.

Methods of statistical data analysis

Statistical data analysis was performed using the R programming language and RStudio software with the following packages: tidyverse, tidymodels, ggplot2, ggpubr, googlesheets4, tidymodels, and rstatix. Normality of data distribution was assessed using the Shapiro — Wilk and Kolmogorov — Smirnov tests, along with skewness and kurtosis analyses, and visualized with QQ plots and distribution histograms. Both parametric and non-parametric statistical methods were utilized for data analysis. Quantitative data were presented as mean (M) ± standard deviation (SD) or as median (Me) with interquartile ranges (25th and 75th percentiles). Categorical data were presented as counts (N) and percentages (%).

The Kruskal — Wallis test was used for group comparisons, followed by post hoc analysis using the Mann — Whitney U test. For categorical variables, contingency tables were created and analyzed using the chi-squared test with Yates' correction. For groups with fewer than five participants, Fisher's exact test was applied, followed by post hoc analysis with Holm's correction for multiple comparisons.

Statistical hypotheses were tested at a significance level of $p < 0.05$, with null hypotheses rejected at this threshold.

Results

The clinical characteristics of the patients included in the analysis are presented in Table 2. The mean age of the patients was 68.0 ± 9.8 years. The study included nearly twice as many women as men: 48 (63 %) vs. 28 (37 %). Almost all patients had abdominal obesity, with a mean body mass index (BMI) of 34.00 ± 7.42 kg/m^2 and an average waist circumference of 114.8 ± 18.8 cm.

All patients enrolled in the study had stage 2B CHF, with 24 (31.6 %) in the decompensated stage. Hypertension was present in 72 (97 %) patients, exertional angina — in 28 (37 %) patients, post-myocardial infarction cardiosclerosis — in 26 (34 %) patients, atrial fibrillation — in 43 (57 %) patients, a history of stroke — in 11 (14 %) patients, and diabetes mellitus — in 19 (25 %) patients. The diagnosis of sarcopenia was confirmed in 52 (68.4 %) patients.

Cluster analysis using the k-means method was performed to group patients based on their clinical characteristics. The "elbow method" was employed to determine the optimal number of clusters, which indicated that dividing the dataset into four clusters was most appropriate. After determining the number of clusters, the data were optimally distributed without any identified outliers.

The centroids obtained from the clustering process are presented in Figure 1. These centroids represent the mean values of the analyzed characteristics within each cluster, providing insights into the common features of patients in each group. Figure 1 uses two colors to differentiate the characteristics: pink represents values below the average, while blue indicates higher-than-average values. This visual distinction enables an intuitive comparison of clinical parameter levels across clusters.

Figure 1 illustrates four bar charts representing the centroids of the four distinct clusters derived from the k-means cluster analysis. Each centroid reflects the mean values of the variables used for clustering. The charts are presented as horizontal bars, with each bar corresponding to a specific short-chain fatty acid (SCFA). The length of the bar indicates the average value of the variable within the cluster. The direction of the bar relative to the vertical line at zero represents the deviation of the variable's value from the baseline level.

Cluster 1

The first cluster encompasses a group of male patients (18.4 % of the total study population) whose average age was below the overall sample mean. The

Table 2. Clinical characteristics of the group (n = 76)
Таблица 2. Клиническая характеристика группы (n = 76)

Parameter ***Параметр***	**Mean** ***Средние***	**SD**	**Median** ***Медиана***	**Min**	**Max**	**25th percentile** ***25-й процентиль***	**75th percentile** ***75-й процентиль***
Age, years *Возраст, годы*	67.97	9.77	69.5	61.0	74.0	45.0	99.0
Height, cm *Рост, см*	164.6	9.64	164.0	157.0	171.25	143.0	186.0
Weight, kg *Вес, кг*	92.72	24.283	89.0	78.0	103.0	35.0	196.0
BMI, kg/m^2 *ИМТ, кг/м^2*	34.0	7.4	33.35	29.08	37.9	17.1	59.8
Office SBP, mmHg *Офисное САД, мм рт. ст.*	131.9	14.0	130.0	120.0	140.0	100.0	185.0
Office DBP, mmHg *Офисное ДАД, мм рт. ст.*	81.8	9.8	80.0	75.0	90.0	60.0	100.0
Heart rate, bpm *ЧСС, уд./мин*	73.2	13.9	70.0	64.0	80.0	45.0	110.0
Respiratory rate, breaths/min *Частота дыхания, дых./мин*	17.5	1.8	17.0	16.0	18.0	15.0	25.0
SARC-F questionnaire, scores *Опросник SARC-F, баллы*	3.9	2.7	4.0	2.0	5.0	0.0	10.0
LVEF, % *ФВ ЛЖ, %*	47.7	14.6	52.0	38.0	55.0	18.0	76.0
AST, U/L *АСТ, Ед./л*	31.3	23.0	25.0	13.6	45.0	16.9	32.6
ALT, U/L *АЛТ, Ед./л*	31.6	25.2	23.8	11.0	46.0	16.7	31.6
Glucose, mmol/L *Глюкоза, ммоль/л*	6.64	1.69	6.2	3.12	12.5	5.4	7.42
Cholesterol, mmol/L *Холестерин, ммоль/л*	4.43	1.22	4.3	1.71	7.67	3.56	5.39

Note: *SD — standard deviation; BMI — body mass index; SBP — systolic blood pressure; DBP — diastolic blood pressure; LVEF — left ventricular ejection fraction; ALT — alanine aminotransferase; AST — aspartate aminotransferase.*
Примечание: *SD (standard deviation) — стандартное отклонение; ИМТ — индекс массы тела; САД — систолическое артериальное давление; ДАД — диастолическое артериальное давление; ЧСС — частота сердечных сокращений; ФВ ЛЖ — фракция выброса левого желудочка; АЛТ — аланинаминотрансфераза; АСТ — аспартатаминотрансфераза.*

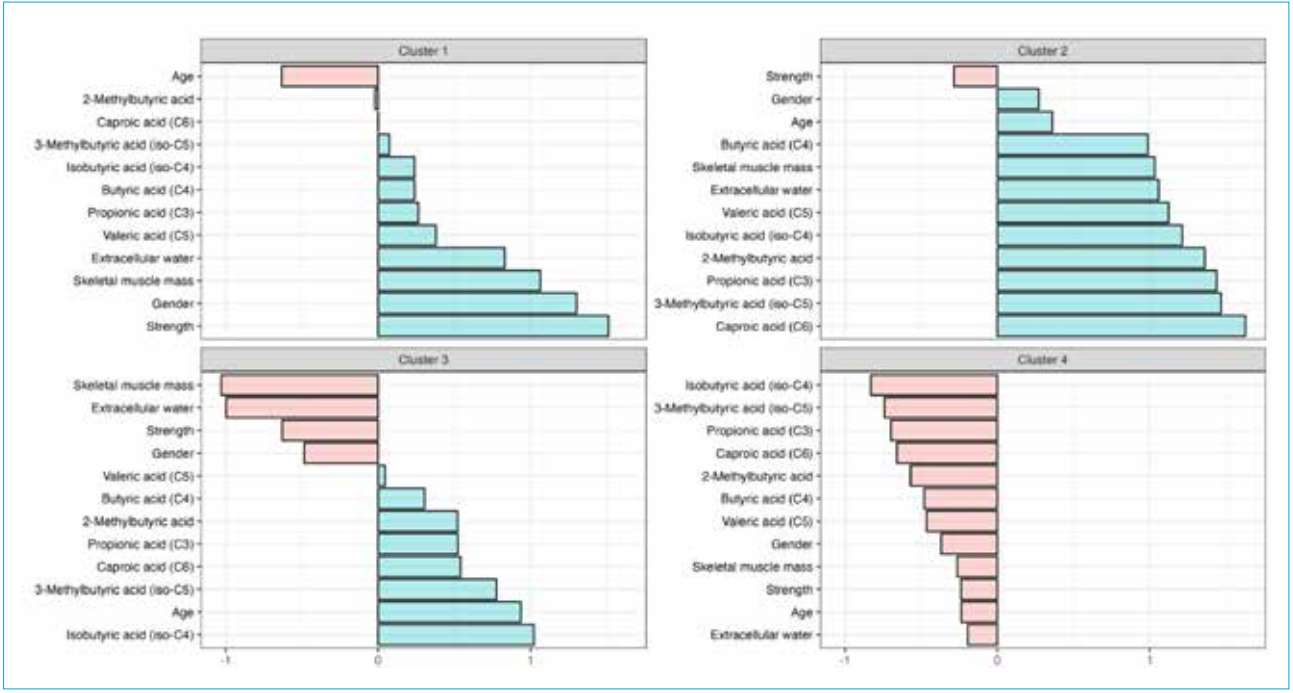

Figure 1. Distribution of centroids in each cluster

Рисунок 1. Распределение центроидов в каждом кластере

mean age of this group was 61.5 (57.5–67.8) years, identifying it as a relatively younger cohort among CHF patients. The clinical profile of these patients was characterized by symptoms such as dyspnea and lower limb edema.

The mean NTproBNP level in this cluster was 1488.4 (1109.6–2681.3) pg/mL. Bioimpedance analysis revealed an average extracellular water content of 19.9 (18.1–24/0) liters, suggesting fluid balance disturbances associated with heart failure.

Assessment of muscle mass showed no signs of sarcopenia in any of the patients in this cluster. This was corroborated by the skeletal muscle mass index (SMMI), which on average deviated only by –7.1 % from the norm (with a variation from –15.0 % to 12.2 %). Handgrip strength, reflecting overall muscle strength, averaged 44.2 (41.1–71.5) kg, indicating good muscular function.

The "Chair Stand" test was completed in 13 seconds or less by this group, demonstrating no significant impairment in muscle function: 15 % of participants completed the test in under 11 seconds, reflecting high levels of physical activity and strength. The Short Physical Performance Battery (SPPB) test yielded an average score of 8 out of 12, with a range from 7 to 10 points. This suggests that reduced muscle function and physical activity were observed in only 29 % of patients in this cluster.

The analysis of SCFA levels in the blood of patients in Cluster 1 revealed that the level of C5 was the lowest among the studied SCFAs, whereas C4 exhibited the highest levels (Table 3). Based on the measured concentrations, the following order of SCFA levels was established for this cluster, ranked from lowest to highest: C5, C6, αC5, C3, βC5, iC4, and C4. This relationship among SCFA levels can be expressed numerically as a following proportion providing a quantitative assessment of the differences in SCFA concentrations:

$$C5/C6/\alpha C5/C3/\beta C5/iC4/C4 = 1 : 3 : 10 : 14 : 18.5 : 18 : 35.$$

For example, the level of C4 was 35 times higher than that of C5, and similar comparisons apply to the other SCFAs. This specific SCFA profile observed in patients of Cluster 1 was designated as "Type 1".

Cluster 2

The second cluster included 10 patients, making up 13.2 % of the total study sample, and is characterized by predominantly elderly participants with an average age of 71.0 (70.0–74.8) years. The gender distribution of the group was balanced, with 50 % men and 50 % women. Similar to patients in Cluster 1, those in Cluster 2 presented with typical symptoms of heart failure, such as dyspnea and lower limb edema. Approximately half of them also reported fatigue and reduced exercise tolerance.

The average NTproBNP level in Cluster 2 was 4559.4 (1860.5–5464.1) pg/mL, the highest among all studied groups. These values may indicate a more severe form of CHF in patients within this cluster. The extracellular water volume, measured by bioimpedance analysis, was also high, with an average of 21.2 (18.7–26.1) liters.

Table 3. Concentration of short-chain fatty acids in blood plasma
Таблица 3. Концентрация короткоцепочечных жирных кислот в плазме крови

Параметр *Parameter*	Кластер 1 *Cluster 1*	Кластер 2 *Cluster 2*	Кластер 3 *Cluster 3*	Кластер 4 *Cluster 4*	*p*
Propanoic acid (C3), ng/mL *Пропановая кислота (C3), нг/мл*	5930 [4580; 6382.5]	7390 [6537.5; 9310]	6580 [4790; 6905]	2890 [2580; 3370]	0.0001
Butanoic acid (C4), ng/mL *Бутановая кислота (C4), нг/мл*	15050 [12525; 18200]	18650 [14950; 21950]	16400 [10100; 20850]	10600 [9220; 12600]	0.0003
Isobutanoic acid (iC4), ng/mL *Изобутановая кислота (iC4), нг/мл*	7785 [3942.5; 10325]	13250 [12500; 14875]	10800 [9900; 14150]	1140 [860; 1440]	0.0001
Pentanoic acid (C5), ng/mL *Пентановая кислота (C5), нг/мл*	472 [368.2; 551.2]	642.5 [553.8; 804.5]	439 [348; 514.5]	314 [277; 433]	0.0001
2-methylbutanoic acid (αC5), ng/mL *2-метилбутановая кислота (αC5), нг/мл*	4200 [2072.5; 7972.5]	10600 [9262.5; 14350]	8180 [5085; 10750]	650 [328; 1330]	0.0001
3-methylbutanoic acid (βC5), ng/mL *3-метилбутановая кислота (βC5), нг/мл*	7885 [1720; 10375]	17180 [14825; 23825]	13600 [10800; 18100]	251 [205; 402]	0.0001
Hexanoic acid (C6), ng/mL *Гексановая кислота (C6), нг/мл*	1140 [839; 1272.5]	2285 [1880; 3642.5]	1330 [1185; 1885]	500 [329; 578]	0.0001

No signs of sarcopenia were observed in Cluster 2. In men, the skeletal muscle mass index (SMMI) was reduced by 6.93 % relative to the norm (ranging from a decrease of –12.5 % to an increase of 23.2 %). In women, however, the reduction was significantly higher at 29 % (with a range from 10.6 % to 91.6 %).

The average handgrip strength for men was 30.6 (30.3–31.1) kg, while for women it was 21.8 (16.7–25.1) kg. In the "Chair Stand" test, patients in this cluster performed worse than those in Cluster 1, as they took more than 15 seconds to complete the task. Additionally, the results of the SPPB test, which assesses overall physical activity and functionality, showed a decline in muscle function, with an average score of 6 out of 12, ranging from 3 to 7.5 points.

Patients in Cluster 2 exhibited elevated levels of SCFAs compared to the overall patient sample (Table 3). These levels were ranked in increasing order, starting with C5 and ending with C4. The SCFA levels can be expressed as proportions, where each subsequent number represents how many times higher the level of the given acid is compared to C5:

C5/C6/C3/αC5/iC4/βC5/C4 =
1 : 3 : 10 : 15 : 19 : 24.5 : 27.

This specific pattern of SCFA levels observed in Cluster 2 patients was designated as "Type 2".

Cluster 3

The third cluster included 15 patients, accounting for 19.7 % of the total sample, with a predominance of women (13 patients; 87 % of this group). This cluster represents the oldest age group in the study, with a median age of 75.0 (72.5–80.5) years.

Patients in Cluster 3 predominantly reported complaints of general weakness, fatigue, and reduced exercise tolerance. The mean NTproBNP level in this group was 685.0 (489.4–1406.3) pg/mL, indicating less severe CHF compared to Cluster 2. The extracellular water volume was 13.6 (12.6–14.8) liters, suggesting better control of fluid balance in this group compared to previous clusters.

All patients in Cluster 3 were diagnosed with sarcopenia. Among men, the median reduction in skeletal muscle mass index (SMMI) was –53.2 % (ranging from –58.8 % to –47.7 %), indicating a significant loss of muscle mass. In women, the reduction in SMMI was –22.1 % (with a range from –32.3 % to –8.7 %), also confirming sarcopenia, albeit less pronounced than in men.

Handgrip strength averaged 39.3 (34.65–43.5) kg in men and 17.7 (10.6–19.8) in women, reflecting substantial muscle weakness, particularly in women. The "Chair Stand" test was completed in more than 15 seconds, similar to the performance of Cluster 2 patients, indicating delayed execution of this task.

The SPPB test, assessing physical activity and functional ability, yielded an average score of 5.5 out of 12, with a range of 4 to 7 points, confirming reduced physical function. Notably, 80 % of patients in this cluster (12 individuals) suffered from severe sarcopenia.

The distribution and hierarchy of SCFA levels in Cluster 3 align precisely with those observed in Cluster 2:

C5/C6/C3/αC5/iC4/βC5/C4 =
1 : 2 : 11.5 : 14 : 19 : 24 : 29.

This identical SCFA distribution underscores the universality of the "Type 2" profile in reflecting the metabolic characteristics of patients in both clusters. Consequently, the SCFA profile of Cluster 3 can be classified as belonging to "Type 2", based on its metabolic similarity to the profile observed in Cluster 2.

Cluster 4

The fourth cluster is the largest group, consisting of 37 patients, which accounts for 48.7 % of the total study population. The mean age of patients in this cluster was 66 (59–70) years. The majority of the patients in this cluster were women, with their number reaching 30 (81 % of the cluster's participants).

The mean NTproBNP level in Cluster 4 was 2038.5 (1530–2547) pg/mL. The average extracellular

water volume was 16.6 (14.9–19.1) liters, indicating varying degrees of fluid balance disturbances.

Signs of sarcopenia were identified in all patients within this cluster, as evidenced by reductions in muscle mass, strength, and function. The reduction in the skeletal muscle mass index (SMMI) among men was –13.4 % on average (ranging from –23.0 % to –11.4 %), while for women, the reduction averaged –10.1 % (with a range from –23.3 % to –1.63 %). The average handgrip strength for men was 32.8 (31.9–35.6) kg, while for women it was 23.75 (21.2–26.2) kg. Similar to participants in Clusters 2 and 3, Cluster 4 patients required more than 15 seconds to complete the "Chair Stand" test, indicating reduced muscle strength. The results of the Short Physical Performance Battery (SPPB) test showed an average score of 9 out of 12, with a range from 8 to 11 points. Only 22 % of patients in this cluster exhibited reduced muscle function and physical activity limitations.

In Cluster 4, SCFA levels were noticeably lower than the average across the entire study sample (Table 3). The SCFA levels in this cluster were ranked in the following order, from βC5 to C4, as reflected in the proportion:

$$\beta C5/C5/C6/\alpha C5/iC4/C3/C4 = 1.5 : 2 : 3 : 4 : 7 : 18 : 65.$$

This proportion illustrates the relative concentrations of the acids, where, for example, the level of C4 is 65 times higher than that of βC5. This specific SCFA distribution identified in Cluster 4 patients was designated as "Type 3".

Post-hoc analysis

A post-hoc analysis was conducted to identify differences between the identified clusters, focusing on the presence of sarcopenia, fluid retention levels, and SCFA concentrations.

In the initial analysis, patients were divided into groups based on the presence or absence of CHF decompensation and sarcopenia. The results showed that patients in Clusters 1 and 2 were in a state of CHF decompensation. However, fluid retention levels did not differ significantly between these clusters: 19.9 (18.1; 24.0) liters — in Cluster 1, 21.2 (18.7–26.1) liters — in Cluster 2; $p = 0.66$.

Additionally, none of the clusters exhibited sarcopenia, as indicated by the skeletal muscle mass index (SMMI): 11.6 (10.7–12.7) kg/m^2 — in Cluster 1, 12.9 (10.3–15.5) kg/m^2 — in Cluster 2; $p = 0.348$.

These results suggest similar profiles for fluid retention and muscle mass in patients with CHF decompensation across these two clusters. Further analysis is needed to explore differences in SCFA levels and their potential implications.

Patients in Clusters 3 and 4 were in different stages of CHF compensation, with more compensated patients assigned to Cluster 3. Extracellular fluid levels were significantly lower in Cluster 3: 13.6 (12.6–14.8) liters vs. 16.6 (14.9–19.1) liters in Cluster 4; $p = 0.000355$.

Both clusters included patients with sarcopenia. The median skeletal muscle mass index (SMMI) was significantly lower in Cluster 3: 7.2 (6.6–7.9) kg/m^2 vs. 8.7 (7.7–9.5) kg/m^2 in Cluster 4; $p = 0.00062$. It indicated that more severe cases of sarcopenia were observed in Cluster 3.

Only patients in Cluster 1 retained normal muscle strength. Comparisons between Cluster 1 and the others showed statistically significant differences: $p = 0.000632$ — for Clusters 1 and 2, $p = 0.000186$ — for Clusters 1 and 3, $p = 0.00000206$ — for Clusters 1 and 4.

However, muscle strength reduction among patients in Clusters 2, 3, and 4 did not show statistically significant differences: $p = 0.288$ — for Clusters 2 and 3, $p = 0.876$ — for Clusters 2 and 4.

Patients in Cluster 3 exhibited more pronounced muscle strength reduction compared to Cluster 4 ($p = 0.009$), highlighting the greater severity of sarcopenia and its impact on muscle strength in Cluster 3.

Pairwise comparisons of SCFA levels among the identified clusters were conducted (Fig. 2). The results revealed the following patterns of inter-cluster differences:

- Clusters 1 and 4 vs. Clusters 2 and 4: Statistically significant differences were identified in the levels of all studied SCFAs. Differences between Clusters 2 and 4 were more pronounced ($p = 0.00001$).
- Clusters 1 and 2 vs. Clusters 3 and 4: Significant differences were observed in the levels of iC4, C3, αC5, βC5, and C6. More substantial differences were found between Clusters 3 and 4 ($p = 0.00001$). However, the levels of C4 and C5 did not show significant differences ($p = 0.06$).

This visualization provides a clear comparison of the SCFA profiles across the clusters, highlighting the unique metabolic characteristics of each group.

Figure 2 presents the results of statistical comparisons of SCFA levels between the four patient clusters. Each column on the chart represents a pairwise comparison between two clusters (e.g., "1 vs. 2", "1 vs. 3", etc.), and each row corresponds to a specific SCFA (e.g., butyric acid, pentanoic acid, etc.). Within each cell of the chart, points indicate the p-value for the comparison of a specific SCFA level between two clusters. The numerical values adjacent to the points display the precise p-values, reflecting the likelihood of error in rejecting the null hypothesis (i.e., no difference

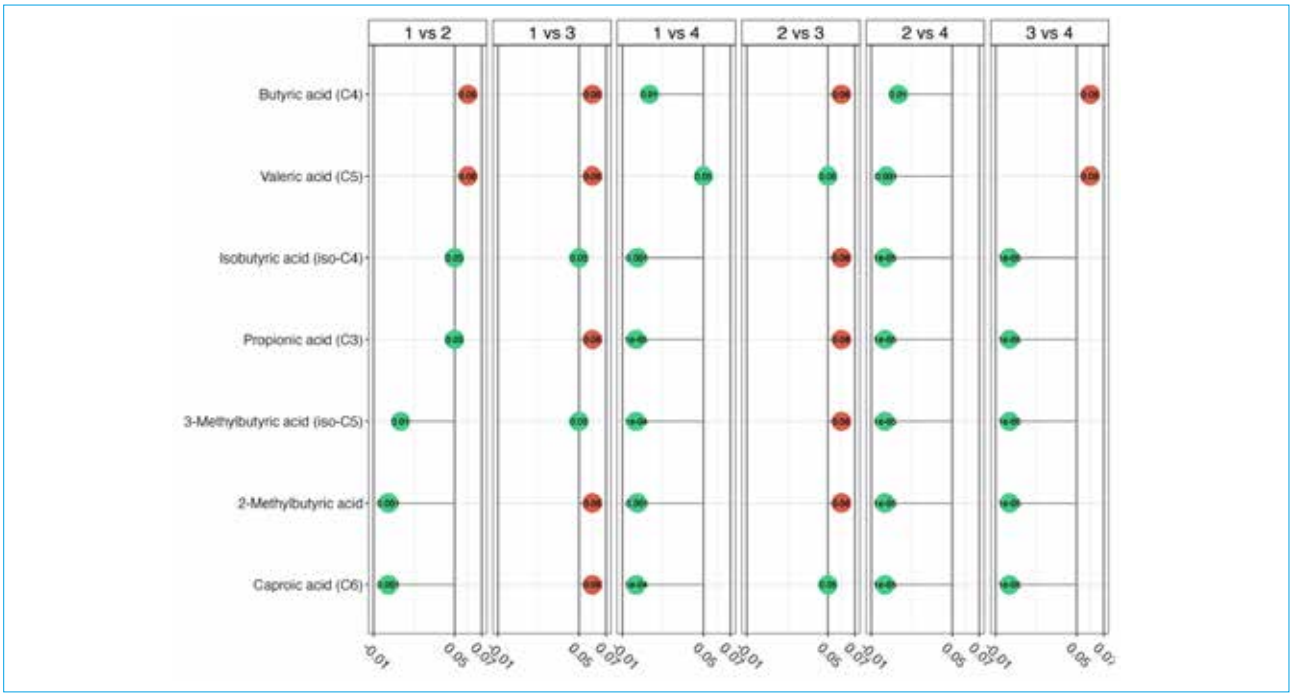

Figure 2. Post-hoc analysis

Рисунок 2. Апостериорный анализ

between groups). Green points indicate non-significant results ($p > 0.05$), suggesting no statistically significant differences in the SCFA levels between the compared clusters. Red points denote statistically significant results ($p < 0.05$), indicating meaningful differences in the SCFA levels between the clusters.

Discussion

Our findings demonstrate that the levels of short-chain fatty acids (SCFAs) in the plasma of patients with chronic heart failure (CHF) and sarcopenia are significantly elevated compared to control values. This elevation may be attributed to compromised intestinal barrier integrity. Increased intestinal permeability in CHF patients, as reported by A. Sandek et al. [10, 11], is associated with intestinal wall hypoxia and edema, which lead to enhanced permeability and elevated SCFA levels in peripheral blood.

These alterations likely reflect a deterioration of the intestinal barrier function, supported by the high levels of butyrate and pentanoic acid observed in the blood of these patients. This suggests a potential link between intestinal barrier dysfunction and metabolic disturbances in CHF, emphasizing the role of SCFAs as possible biomarkers for disease progression and gut permeability.

It is well-established that butyrate plays a crucial role in maintaining intestinal barrier integrity and exhibits anti-inflammatory properties [12]. Elevated plasma levels of butyrate may indicate increased translocation through a compromised intestinal wall, aligning with the observed barrier dysfunction in CHF patients with sarcopenia. Additionally, high concentrations of pentanoic acid are likely associated with increased intestinal permeability and alterations in the gut microbiota. These changes may contribute to disruptions in metabolic processes and the progression of sarcopenia [13, 14]. This suggests that both butyrate and pentanoic acid could serve as markers of intestinal and metabolic disturbances in this patient population, highlighting the interconnected roles of gut health and systemic metabolic regulation.

The gut microbiota and its metabolites, including SCFAs, play a critical role in the pathogenesis of sarcopenia and CHF [15, 16]. Dysbiosis, particularly the reduction in beneficial bacteria and an increase in opportunistic species, is associated with decreased microbial diversity and a deterioration of metabolic status [17, 18]. Studies indicate that changes in SCFA levels, such as butyrate and propionate, may promote inflammatory processes, leading to muscle loss and reduced physical activity [19]. For example, J. Yin et al. [3] highlighted the significant role of inflammation in the pathogenesis of sarcopenia, which aligns with our findings.

A key aspect of our results is the heterogeneity observed among patients, as demonstrated by

cluster analysis. The clusters exhibited distinct SCFA profiles and clinical characteristics, potentially reflecting different disease stages and emphasizing the need for individualized treatment approaches. Patients in clusters with high levels of butyrate and pentanoic acid are likely to experience more pronounced dysbiosis and inflammatory processes, necessitating targeted therapeutic strategies [15, 16].

Thus, our findings highlight the importance of a comprehensive approach to the treatment of patients with CHF and sarcopenia. This approach should include interventions aimed at correcting gut microbiota composition and improving intestinal barrier function to reduce SCFA levels and minimize inflammatory processes. Further research is required to better understand the mechanisms underlying the interactions between the microbiota, gut metabolites, and the pathogenesis of sarcopenia. Such insights could facilitate the development of novel therapeutic strategies [3, 16, 19].

Conclusions

Differences in SCFA levels between clusters suggest potential links between SCFA metabolism, heart failure, and the progression of sarcopenia. These differences may serve as biomarkers for identifying patients at high risk of sarcopenia and underscore the need for an individualized treatment approach, including the correction of SCFA metabolism and gut microbiota composition.

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