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Anatomical and Molecular Insights into Sarcopenia in Liver Cirrhosis: A CT-Based and Biomarker Case-Control Study

Costel-George Gherghescu[1,2#], Radu Andrei Baz[2,3#], Constantin Dina[3#*], Andrei Dumitru[3,4#*], Georgeta Camelia Cozaru[5#], Mihaela Manea[5#], Ioana Popescu[3,4#], Ana-Maria Grigorescu[3#], Stere Popescu[3,4#], and Eugen Dumitru[3,5,6,7#]

[1]PhD School of Medicine, Ovidius University, Constanta, Romania
[2]Clinical Laboratory of Radiology and Medical Imaging, Sf. Apostol Andrei Emergency County Hospital, Constanta, Romania
[3]Faculty of Medicine, Ovidius University, Constanta, Romania
[4]Department General Surgery, Sf. Apostol Andrei County Emergency Hospital, Constanta, Romania
[5]Department of Gastroenterology, Sf. Apostol Andrei Emergency County Hospital, Constanta, Romania
[6]Research Center for the Morphological and Genetic Study in Malignant Pathology, Ovidius University, Constanta, Romania
[7]Academy of Romanian Scientist, Bucharest, Romania

***Corresponding author:**
Andrei Dumitru, MD
Department of Gastroenterology,
Sf. Apostol Andrei Emergency
County Hospital
900591 Constanta Romania
E-mail:dr.andreidumitru@gmail.com
Constantin Dina, MD
Department of Anatomy,
Ovidius University of Constanta
1 University Street, 900470
E-mail: constantin-dina@365.univ-ovidius.ro

[#]All authors share equal contribution.

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Rezumat

Aspecte anatomice și moleculare ale sarcopeniei în ciroza hepatică: studiu caz-control bazat pe tomografia computerizată și biomarkeri serici

Introducere: Sarcopenia reprezintă o complicație frecventă şi un factor prognostic negativ la pacienții cu ciroză hepatică, fiind asociată cu scăderea masei şi funcției musculare, precum şi cu o mortalitate crescută. Deşi tomografia computerizată este considerată standardul de referință pentru evaluarea masei musculare, există un interes tot mai mare pentru identificarea unor biomarkeri serici care să permită diagnosticul precoce şi monitorizarea evoluției bolii. Scopul studiului a fost evaluarea valorii diagnostice a osteonectinei, fragmentului C-terminal al agrinei (CAF), propeptidei N-terminale a procolagenului de tip III (P3NP) şi miostatinei la pacienții cu ciroză hepatică şi sarcopenie, în corelație cu parametrii imagistici determinați prin CT.
Material şi Metode: A fost realizat un studiu observațional prospectiv de tip caz-control, care a inclus 60 de participanți, dintre care 30 de pacienți cu ciroză hepatică (cu sau fără carcinom hepatocelular) şi sarcopenie şi 30 de subiecți martor sănătoşi. Diagnosticul sarcopeniei s-a bazat pe criteriile EWGSOP2 şi pe determinarea indicelui masei musculare scheletice (SMI) şi a indicelui muşchiului psoas (PMI) prin tomografie computerizată. Concentrațiile serice ale osteonectinei, CAF, P3NP şi miostatinei au fost determinate prin metoda ELISA. Analiza statistică a inclus teste parametrice şi neparametrice, precum şi analiza corelațiilor Pearson.
Rezultate: Pacienții cu ciroză hepatică şi sarcopenie au prezentat valori semnificativ crescute ale osteonectinei, CAF, P3NP şi miostatinei comparativ cu lotul martor ($p<0,001$ pentru toate comparațiile). Indicele masei musculare scheletice a fost semnificativ redus la pacienții cu ciroză, confirmând pierderea masei musculare. Analiza corelațională a evidențiat asocieri pozitive între osteonectină şi CAF ($r=0,441$; $p<0,001$), osteonectină şi P3NP ($r=0,313$; $p=0,016$),

osteonectină şi miostatină (r=0,444; p<0,001), precum şi o corelație puternică între CAF şi miostatină (r=0,882; p<0,001). Nu s-au identificat corelații semnificative între biomarkeri şi etiologia virală sau clasa Child-Pugh.
Concluzii: Determinarea simultană a osteonectinei, CAF, P3NP şi miostatinei, împreună cu evaluarea imagistică prin tomografie computerizată, poate îmbunătăți diagnosticul sarcopeniei asociate cirozei hepatice şi poate contribui la identificarea precoce a pacienților cu risc crescut de deteriorare musculară. Sunt necesare studii prospective multicentrice, pe loturi mai mari, pentru validarea utilității clinice a acestor biomarkeri în practica medicală curentă.

Cuvinte cheie: sarcopenie, ciroză hepatică, biomarkeri serici, osteonectină, fragment C-terminal al agrinei, P3NP, miostatină, tomografie computerizată, indicele masei musculare scheletice, carcinom hepatocelular

Abstract

Background: Sarcopenia is a common complication and an important negative prognostic factor in patients with liver cirrhosis, being associated with reduced muscle mass and function, as well as increased mortality. Although computed tomography (CT) is considered the gold standard for assessing muscle mass, there is growing interest in identifying serum biomarkers that may facilitate the early diagnosis and monitoring of disease progression. The aim of this study was to evaluate the diagnostic value of osteonectin, the C-terminal agrin fragment (CAF), the N-terminal propeptide of type III procollagen (P3NP), and myostatin in patients with liver cirrhosis and sarcopenia, in correlation with CT-derived imaging parameters.
Materials and Methods: A prospective observational case–control study was conducted, including 60 participants: 30 patients with liver cirrhosis (with or without hepatocellular carcinoma) and sarcopenia, and 30 healthy control subjects. Sarcopenia was diagnosed according to the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) criteria, using the skeletal muscle index (SMI) and psoas muscle index (PMI) measured by computed tomography. Serum concentrations of osteonectin, CAF, P3NP, and myostatin were determined using enzyme-linked immunosorbent assay (ELISA). Statistical analyses included parametric and non-parametric tests, as well as Pearson correlation analysis.
Results: Patients with liver cirrhosis and sarcopenia exhibited significantly higher serum levels of osteonectin, CAF, P3NP, and myostatin compared with healthy controls ($p < 0.001$ for all comparisons). The skeletal muscle index was significantly lower in cirrhotic patients, confirming the presence of muscle wasting. Correlation analysis demonstrated positive associations between osteonectin and CAF ($r = 0.441$, $p < 0.001$), osteonectin and P3NP ($r = 0.313$, $p = 0.016$), osteonectin and myostatin ($r = 0.444$, $p < 0.001$), as well as a strong correlation between CAF and myostatin ($r = 0.882$, $p < 0.001$). No significant associations were observed between biomarker levels and viral etiology or Child–Pugh class.
Conclusions: Simultaneous assessment of osteonectin, CAF, P3NP, and myostatin, combined with computed tomography-based imaging evaluation, may improve the diagnosis of sarcopenia associated with liver cirrhosis and facilitate the early identification of patients at increased risk of muscle deterioration. Larger multicenter prospective studies are warranted to validate the clinical utility of these biomarkers in routine medical practice.

Key words: sarcopenia, liver cirrhosis, biomarkers, osteonectin, myostatin, C-terminal agrin fragment, P3NP, muscle wasting, fibrosis, computed tomography

Introduction

Sarcopenia represents a major determinant of frailty in the elderly population and is defined by the progressive loss of skeletal muscle mass accompanied by reduced muscle strength and/or impaired physical performance (1,2). The assessment of muscle mass relies on imaging modalities such as magnetic resonance imaging (MRI), computed tomography (CT), dual-energy X-ray absorptiometry (DEXA), and bioelectrical impedance analysis (BIA). However, functional tests, including handgrip strength and gait speed, are often influenced by coexisting comorbidities, particularly musculoskeletal and neurological disorders, which are highly prevalent in this population (3). Consequently, increasing attention has been directed toward the identification of circulating molecular biomarkers that may facilitate early diagnosis and improve prognostic stratification in sarcopenia (4).

In the context of liver cirrhosis, sarcopenia has emerged as an independent prognostic factor associated with adverse clinical outcomes. It has been linked to a higher risk of postoperative complications, including infections and the need for mechanical ventilation following liver transplantation, and is reported to

affect up to 65–100% of patients with advanced liver disease (5).

CT is widely regarded as one of the most reliable methods for evaluating skeletal muscle mass and quality, owing to its extensive use in the diagnosis and monitoring of both acute and chronic conditions. A commonly applied approach involves the quantification of the skeletal SMI and PMI at the level of the L3–L4 vertebrae, based on muscle attenuation values ranging from 29 to 150 Hounsfield Units (HU) (6). These measurements are typically obtained by manually delineating regions of interest (ROI) on non-contrast images, as contrast-enhanced scans may artificially increase attenuation values due to the iodophilic properties of the tissue and are therefore not recommended for analysis (7).

Established cut-off values for SMI range between 52–55 cm^2/m^2 in males and 39–41 cm^2/m^2 in females, while PMI thresholds are reported at approximately 6.31 cm^2/m^2 in males and 3.91 cm^2/m^2 in females (8,9). However, the lack of universally accepted reference standards remains a significant limitation in the clinical application of these indices.

The pathogenesis of sarcopenia is complex and multifactorial, involving both intrinsic mechanisms, such as inflammation, apoptosis, autophagy, and alterations in calcium homeostasis, and extrinsic factors, including nutritional deficiencies, reduced physical activity, and endocrine dysregulation. These processes collectively impair myogenesis and promote progressive muscle atrophy (3). Given this biological complexity, no single biomarker is sufficient to accurately capture the full spectrum of sarcopenia. Accordingly, the present study aims to perform a comparative evaluation of circulating biomarkers in both healthy individuals and patients with sarcopenia, with the objective of identifying candidates with potential diagnostic and prognostic utility.

Materials and Methods

Study Design

This prospective observational study included a total of 60 participants evaluated at the County Emergency Clinical Hospital of Constanța, Romania, between January 2022 and December 2023. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Sf. Apostol Andrei County Emergency Clinical Hospital (approval no. 05/04.02.2022).

Study Population and Eligibility Criteria

Participants were eligible if they were admitted during the study period, aged between 39 and 82 years, and had complete clinical and paraclinical data available. The study cohort was divided into two groups: 30 patients diagnosed with liver cirrhosis (+HCC) and sarcopenia, based on the criteria of the European Working Group on Sarcopenia in Older People (EWGSOP), and 30 control subjects without known chronic diseases.

Baseline clinical data, including age, sex, body weight, height, and comorbidities such as diabetes mellitus, chronic obstructive pulmonary disease, and smoking status, were collected from medical records.

Exclusion criteria included patients with severe immobilization, autoimmune diseases, uncontrolled endocrine disorders, and conditions significantly affecting overall health status, such as malignancies, advanced heart failure, uremia, or septic shock.

Biomarker Assessment and Imaging Analysis

Venous blood samples were collected from all participants, and biomarker measurements were performed immediately after collection. Remaining plasma samples were stored at −80°C for subsequent analyses. Circulating levels of osteonectin (SPARC), C-terminal agrin fragment (CAF), N-terminal propeptide of type III procollagen (P3NP), and myostatin (MSTN) were determined using enzyme-linked immunosorbent assay (ELISA) kits, in accordance with the manufacturer's protocols.

Muscle mass and quality were assessed using CT scans of the upper abdomen. The skeletal muscle index (SMI) and psoas muscle index (PMI) were measured at the level of the L3 vertebral body on non-contrast images by manually delineating regions of interest (ROI).

The following cut-off values were applied: 52–55 cm^2/m^2 for males and 39–41 cm^2/m^2 for females for SMI, and ≤6.31 cm^2/m^2 for males and ≤3.91 cm^2/m^2 for females for PMI.

Sarcopenia was defined according to the revised European Working Group on Sarcopenia in Older People (EWGSOP2) criteria, integrating both imaging-based muscle assessment and clinical evaluation. Patient selection was performed consecutively during the study period based on predefined inclusion and exclusion criteria.

The presence of ascites was evaluated clinically and radiologically at the time of assessment. Patients with clinically significant or tense ascites were considered unsuitable for reliable bioelectrical impedance-derived body composition measurements because of the potential influence of fluid overload on impedance parameters. In these cases, CT-based skeletal muscle

evaluation, including SMI and PMI, was preferentially used, as these measurements are less affected by extracellular fluid retention.

Body composition parameters, including body weight, body mass index (BMI), visceral fat level, total body water, muscle mass, and body fat percentage, were assessed using a multifrequency bioelectrical impedance analyzer (Tanita BC-401, Tanita Corporation, Tokyo, Japan). Measurements were performed with participants in standing position, barefoot, under standardized conditions, in the morning and after a fasting period of at least 8 hours whenever possible. Participants were instructed to avoid intense physical activity and excessive fluid intake prior to evaluation. All measurements were obtained using the manufacturer's standard protocol. In cases of inconsistent readings, measurements were repeated and the final value was recorded after confirmation of reproducibility.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 25 and Microsoft Office Excel/Word 2021. Categorical variables were expressed as absolute numbers and percentages, and differences between groups were evaluated using Fisher's exact test. When appropriate, Z-tests with Bonferroni correction were applied for post hoc comparisons.

Continuous variables were reported as mean ± standard deviation or median with interquartile range, depending on data distribution, which was assessed using the Shapiro-Wilk test. Normally distributed variables were compared using one-way ANOVA or Welch ANOVA, based on variance homogeneity assessed by Levene's test, followed by Games-Howell post hoc analysis. Non-normally distributed variables were analyzed using the Kruskal-Wallis H test, with Dunn–Bonferroni correction applied for multiple comparisons.

Results

Distribution According to Population Characteristics

The study population was evenly distributed between the control group (49.2%) and patients with HCC and liver cirrhosis (50.8%). A clear predominance of male participants was observed (61.0% vs. 39.0% females) (*Table 1*).

Regarding viral etiology, hepatitis B infection was present in 30.0% of cases, while hepatitis C was identified in 23.3% of patients. Coinfection with hepatitis B and D viruses was relatively uncommon (6.7%).

Assessment of liver disease severity, based on the Child-Pugh classification, revealed that the majority of patients were classified as stage B (50.0%), followed by stage A (33.3%) and stage C (16.7%).

The descriptive analysis revealed a cohort with a mean age of 60 years, predominantly characterized by moderate alterations in both metabolic and nutritional parameters (*Table 2*).

Biomarker analysis revealed markedly elevated mean levels of osteonectin (602.76 ng/mL), CAF (1784.23 pg/mL), and P3NP (5.54 ng/mL), with median values closely aligned with central tendencies. In contrast, myostatin exhibited a notable discrepancy between mean (27.72 ng/mL) and median values (7.65 ng/mL). Inflammatory and metabolic markers further supported a state of systemic dysfunction. Elevated mean C-reactive protein levels (3.46 mg/dL) indicate an underlying inflammatory burden, while reduced albumin levels (mean 2.97 g/dL) suggest impaired hepatic synthetic function and potential malnutrition. Renal function remained relatively preserved, as reflected by stable creatinine values, whereas glycemic levels showed mild elevation. The mean skeletal muscle index (42.48 cm^2/m^2) approaches diagnostic thresholds for sarcopenia, while muscle mass values (47.5 kg) and psoas muscle density (65 HU) indicate reduced muscle quality. Concurrently, increased visceral fat (mean 11.3) and body fat percentage (29.7%) suggest a redistribution toward adiposity.

Additionally, there is a markedly reduced vitamin D levels (mean 11.40 ng/mL). The relatively narrow confidence intervals for most median values indicate acceptable variability; however, wider dispersion

Table 1. Baseline demographic and clinical characteristics of the study population

	Count	Table N %
Group		
Control	30	49.2%
HCC and cirrhosis	30	50.8%
Sex		
Female	23	39.0%
Male	36	61.0%
Hepatitis B		
No	21	70.0%
Yes	9	30.0%
Hepatitis C		
No	23	76.7%
Yes	7	23.3%
Hepatitis B+D		
No	28	93.3%
Yes	2	6.7%
Child-Pugh_class		
A	10	33.3%
B	15	50.0%
C	5	16.7%

Table 2. Descriptive statistics of clinical, biochemical, and body composition parameters in the study population

	Mean	Standard Error of Mean	Median	95.0% Lower CL for Median	95.0% Upper CL for Median
Age (years)	60	1	61	57	66
Osteonectin (ng/mL)	602.76	19.24	576.35	529.63	622.44
C-terminal agrin fragment (pg/mL)	1784.23	169.35	1417.37	1245.20	1711.07
P3NP (ng/mL)	5.54	.12	5.62	5.38	6.01
Myostatin (ng/mL)	27.72	6.05	7.65	6.36	9.26
Insulin-like growth factor 1 (ng/mL)	111.42	4.67	104.67	96.35	118.67
C-reactive protein (mg/dL)	3.46	.53	1.20	.71	2.87
Creatinine (mg/dL)	1.00	.05	.85	.78	1.15
Albumin (g/dL)	2.97	.08	2.90	2.70	3.30
Blood glucose (mg/dL)	103.16	3.42	95.00	93.00	100.00
Vitamin D (ng/mL)	11.40	.69	10.40	8.77	11.30
Psoas muscle density	65	3	67	57	82
Skeletal muscle index (cm^2/m^2)	42.48	1.25	44.00	42.10	48.70
Body weight (kg)	81.2	2.1	81.5	74.9	87.0
Body mass index (kg/m^2)	26.44	.52	25.76	25.40	27.74
Visceral fat level	11.3	.5	11.0	10.5	12.4
Total body water (%)	48.3	.6	48.5	46.0	50.0
Muscle mass (kg)	47.5	.8	48.6	47.5	50.0
Body fat (%)	29.7	.8	28.5	27.5	30.0

observed in selected biomarkers (e.g., CAF, myostatin) highlights inter-individual heterogeneity, which may reflect differences in disease severity or comorbidity burden.

This boxplot in *Fig. 1* illustrates the distribution of osteonectin levels stratified by sex across the control group and patients with liver cirrhosis with or without hepatocellular carcinoma.

The data presented, illustrate the comparison of osteonectin levels across the study groups. The distribution of osteonectin values was non-parametric according to the Shapiro-Wilk test ($p<0.05$). Differences between groups were statistically significant based on the Kruskal–Wallis H test ($p < 0.001$), and post hoc analysis revealed the following: Patients in the control group had significantly lower osteonectin levels (median = 539.9, IQR = 482.7–635) compared to patients with liver cirrhosis, with or without hepatocellular carcinoma (median = 927.8, IQR = 807.7–1244.5) ($p < 0.001$).

The data presented in *Table 3* and *Fig. 2*, illustrate the comparison of CAF levels across the study groups.

The distribution of CAF values was non-parametric

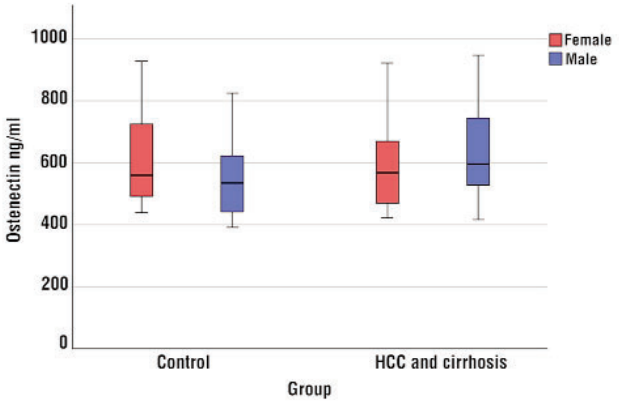

Figure 1. Comparison of osteonectin levels according to the study groups

in all groups according to the Shapiro-Wilk test ($p < 0.05$). Differences between groups were statistically significant based on the Kruskal-Wallis H test ($p < 0.001$), and post hoc analysis showed that patients in the control group had significantly lower CAF levels (median = 1165.5, IQR = 1024.6–1369.4) compared to patients with liver cirrhosis, with or without

Table 3. Comparison of CAF values according to the study groups

Group	Mean ± SD	Median (IQR)	Mean Rank	p*
Control (p<0.001**)	1580.4 ± 1687.6	1165.5 (1024.6-1369.4)	25.78	<0.001
Cirrhosis ± HCC (p<0.001**)	2290 ± 1429.1	1781.3 (1612.6-2388.6)	72.45	

*Kruskal-Wallis H Test, **Shapiro-Wilk Test

Table 4. Comparison of P3NP levels according to the study groups

Group	Mean ± SD	Median (IQR)	Mean Rank	p*
Control (p=0.548**)	5.25 ± 0.86	5.34 (4.6-5.9)	20.28	<0.001
Cirrhosis± HCC (p=0.216**)	7.35 ± 1.2	7.16 (6.3-8.08)	68.88	

*Kruskal-Wallis H Test, **Shapiro-Wilk Test

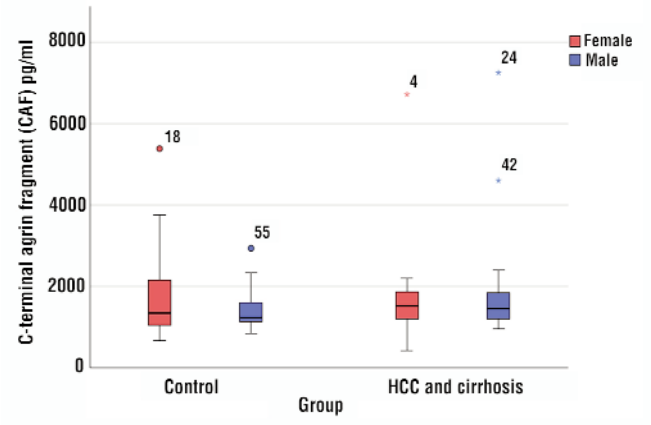

Figure 2. Comparison of CAF levels according to the study groups

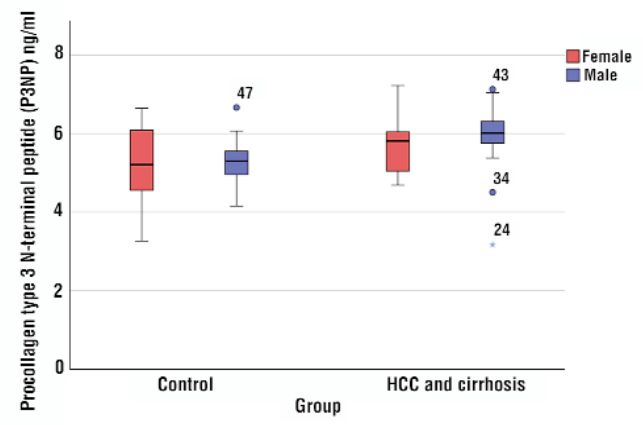

Figure 3. Comparison of P3NP levels according to the study groups

hepatocellular carcinoma (median = 1781.3, IQR = 1612.6-2388.6) (p < 0.001).

The data presented in *Table 4* and *Fig. 3*, illustrate the comparison of P3NP levels across the study groups.

The distribution of P3NP values was non-parametric in all groups according to the Shapiro–Wilk test (p < 0.05). Differences between groups were statistically significant based on the Kruskal–Wallis H test (p < 0.001), and post hoc analysis showed that patients in the control group had significantly lower P3NP levels (median = 5.34, IQR = 4.6–5.9) compared to patients with liver cirrhosis, with or without hepatocellular carcinoma (median = 7.16, IQR = 6.3–8.08) (p < 0.001).

The data presented in *Table 5* and *Fig. 4*, illustrate the comparison of myostatin levels across the study groups.

The distribution of myostatin values was non-parametric in all groups according to the Shapiro-Wilk test (p < 0.05). Differences between groups were statistically significant based on the Kruskal-Wallis H test (p < 0.001), and post hoc analysis showed that patients in the control group had significantly lower

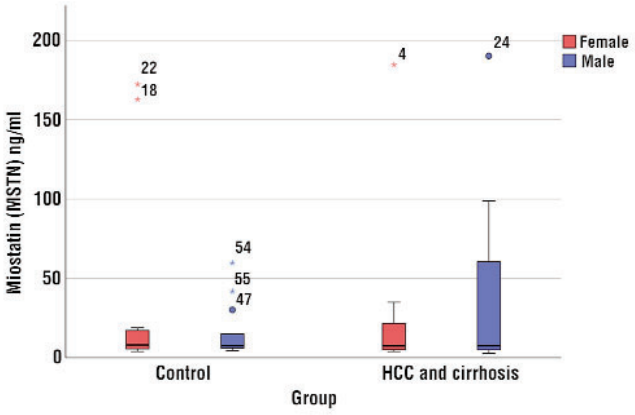

Figure 4. Comparison of myostatin levels according to the study groups

myostatin levels (median = 7.85, IQR = 6.02-15.78) compared to patients with liver cirrhosis, with or without hepatocellular carcinoma (median = 35.86, IQR = 18.18-61.1) (p < 0.001).

Table 6 presents the Pearson correlation coefficients assessing the relationships between viral

Table 5. Comparison of myostatin levels according to the study groups

Group	Mean ± SD	Median (IQR)	Mean Rank	p*
Control (p<0.001**)	20 ± 31.73	7.85 (6.02-15.78)	30.27	<0.001
Cirrhosis ± HCC (p<0.001**)	47.8 ± 45.17	35.86 (18.18-61.1)	70.85	

*Kruskal-Wallis H Test, **Shapiro-Wilk Test

Table 6. Pearson correlation analysis between viral hepatitis status, Child–Pugh class, and serum biomarkers

	Hepatitis B	Hepatitis C	Hepatitis B+D	Child-Pugh class	Ostenectin ng/ml	CAF pg/ml	P3NP ng/ml	MSTN ng/ml
Hepatitis B								
Pearson Correlation	1	-.361*	-.175	.053	-.264	-.301	-.098	-.249
Sig. (2-tailed)		.050	.355	.781	.158	.106	.607	.184
Hepatitis C								
Pearson Correlation	-.361*	1	-.147	.019	.032	.113	.051	.133
Sig. (2-tailed)	.050		.437	.920	.865	.553	.790	.485
Hepatitis B+D								
Pearson Correlation	-.175	-.147	1	.065	.154	-.026	.066	.011
Sig. (2-tailed)	.355	.437		.734	.416	.892	.729	.953
Child-Pugh class								
Pearson Correlation	.053	.019	.065	1	-.072	-.101	-.074	-.213
Sig. (2-tailed)	.781	.920	.734		.705	.596	.699	.259
Ostenectin ng/ml								
Pearson Correlation	-.264	.032	.154	-.072	1	.441**	.313*	.444**
Sig. (2-tailed)	.158	.865	.416	.705		<.001	.016	<.001
CAF pg/ml								
Pearson Correlation	-.301	.113	-.026	-.101	.441**	1	-.051	.882**
Sig. (2-tailed)	.106	.553	.892	.596	<.001		.702	<.001
P3NP ng/ml								
Pearson Correlation	-.098	.051	.066	-.074	.313*	-.051	1	-.222
Sig. (2-tailed)	.607	.790	.729	.699	.016	.702		.092
MSTN ng/ml								
Pearson Correlation	-.249	.133	.011	-.213	.444**	.882**	-.222	1
Sig. (2-tailed)	.184	.485	.953	.259	<.001	<.001	.092	

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

hepatitis types (HBV, HCV, HBV+D co-infection), Child-Pugh class, and serum biomarkers (osteonectin, CAF, P3NP, and MSTN).

Significant negative correlation was observed between Hepatitis B and Hepatitis C ($r = -0.361$, $p = 0.050$), suggesting an inverse distribution of these infections within the study population. No significant correlations were identified between viral hepatitis variables or Child–Pugh class and the evaluated biomarkers ($p > 0.05$), indicating that liver disease etiology and severity were not directly associated with biomarker levels in this cohort.

In contrast, several significant positive correlations were observed among the biomarkers themselves. Osteonectin demonstrated moderate positive correlations with CAF ($r = 0.441$, $p < 0.001$), P3NP ($r = 0.313$, $p = 0.016$), and MSTN ($r = 0.444$, $p < 0.001$). CAF showed a strong positive correlation with MSTN ($r = 0.882$, $p < 0.001$), representing the most robust association identified in the analysis.

Fig. 5 presents a scatterplot matrix depicting the pairwise relationships between clinical variables (Hepatitis B, Hepatitis C, Hepatitis B+D co-infection, Child-Pugh class) and serum biomarkers (osteonectin, CAF, P3NP, and myostatin).

Visual inspection suggests a lack of strong linear relationships between viral hepatitis types or Child-Pugh class and the evaluated biomarkers, as indicated by the dispersed and non-patterned distribution of points. In contrast, moderate positive associations can be observed between certain biomarkers, particularly between osteonectin, CAF, and myostatin, where clustering of values suggests potential interdependence. The relationship between CAF and myostatin appears especially pronounced, supporting the strong correlation identified in the statistical analysis.

Fig. 6 shows the axial native CT images at the level of the L3 vertebral body illustrate the quantitative assessment of skeletal muscle mass using region-of-interest (ROI) segmentation. In image (A), both the total abdominal skeletal musculature and paraspinal muscle groups are delineated, allowing for the calculation of the skeletal muscle index (SMI). In image (B), a focused segmentation of the psoas muscles is performed to determine the psoas muscle index (PMI).

The reduced cross-sectional muscle area observed in both images is indicative of decreased muscle mass, consistent with sarcopenia. The attenuation values within the defined ROIs fall within the expected range for skeletal muscle (29–150 HU),

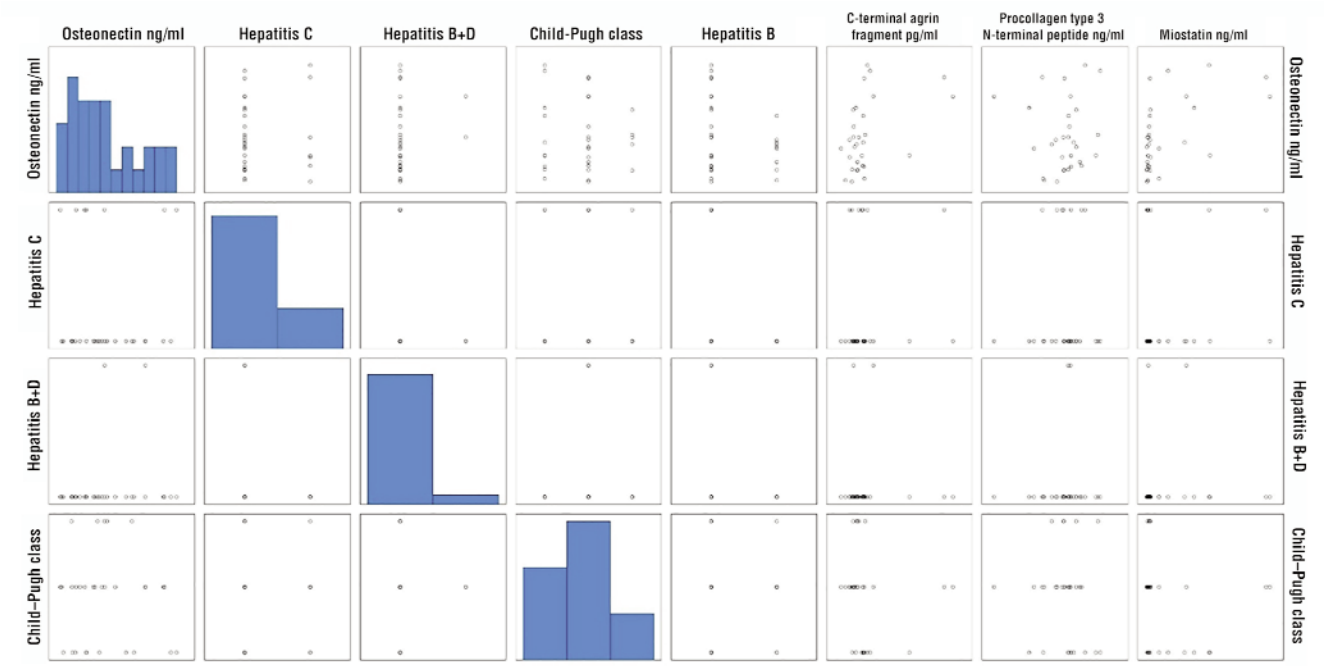

Figure 5. Scatterplot matrix illustrating relationships between clinical variables and serum biomarkers. The diagonal plots illustrate the distribution of each variable, while the off-diagonal panels display scatterplots reflecting potential associations between variables.

supporting accurate tissue characterization.

In image (A) of *Fig. 7*, the contouring includes the entire abdominal skeletal musculature, while in image (B), segmentation is limited to the bilateral psoas muscles for the determination of the PMI.

Compared to normal anatomical expectations, both images reveal a reduced cross-sectional muscle area and diminished muscle bulk, suggestive of sarcopenia. The attenuation values within the segmented regions remain within the typical range for skeletal muscle tissue, supporting the reliability of the measurements.

Table 7 presents the comparison of SMI levels according to the study grops.

This boxplot in *Fig. 8* illustrates the distribution of SMI values stratified by sex across the groups. In both groups, males exhibit higher median SMI values compared to females, reflecting the expected sex-related differences in skeletal muscle mass.

Within the control group, SMI values are generally higher and more homogeneous, whereas in the cirrhosis /HCC group, a downward shift in median values is observed, particularly among

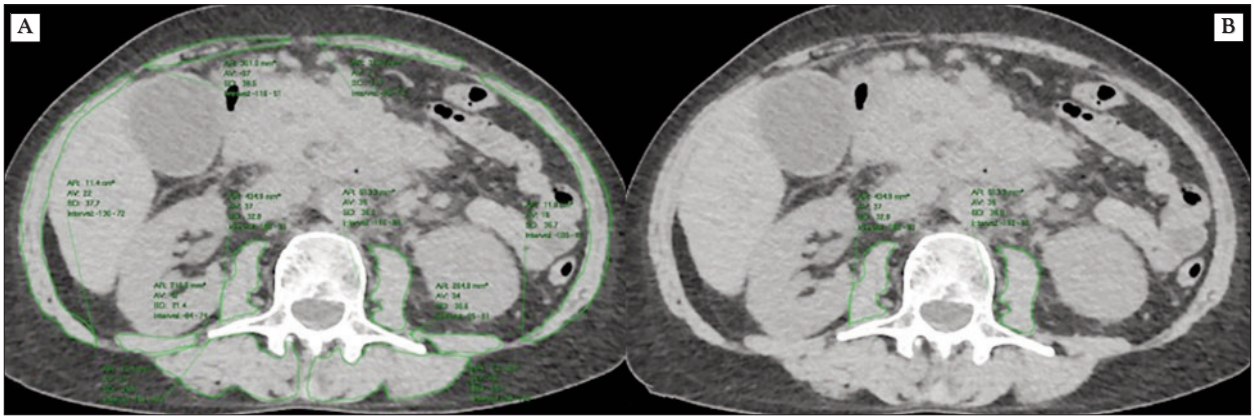

Figure 6. Native axial CT images demonstrating SMI values of 25.56 cm^2/m^2 **(A)** and PMI values of 3.27 cm^2/m^2 **(B)**.

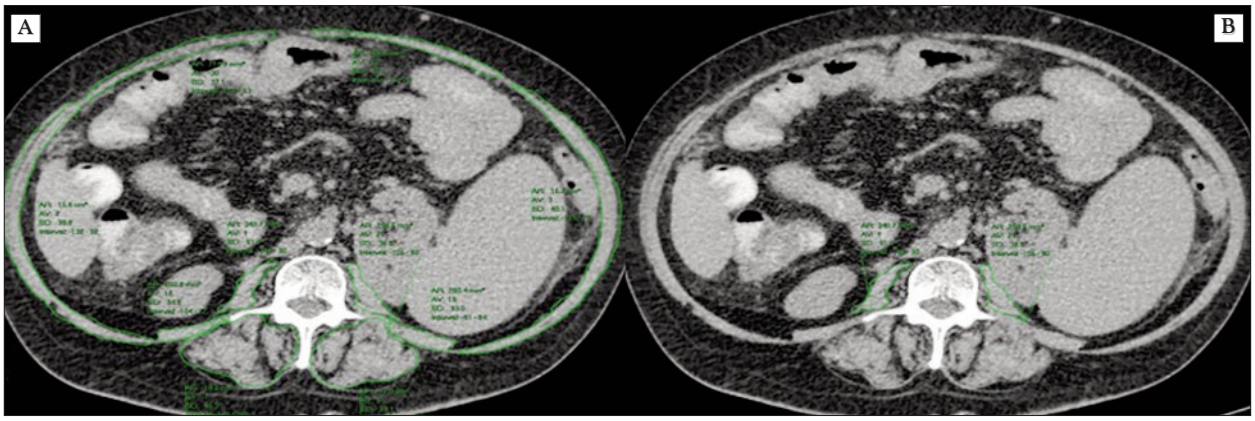

Figure 7. Native axial CT images demonstrating SMI values of 30 cm²/m² (**A**) and PMI values of 2.35 cm²/m² (**B**).

Table 7. Comparison of SMI levels according to the study grops

Group	Mean ± SD	Median (IQR)	Mean Rank	p*
Group (p=0.014**)	46.2 ± 6.2	45.88 (39.7-52.2)	87.95	<0.001
Cirrhosis ± HCC (p<0.001**)	38.1 ± 6.62	41.3 (34.6-42.78)	54.83	

*Kruskal-Wallis H Test, **Shapiro-Wilk Test

females, suggesting a greater degree of muscle mass depletion.

Discussion

Sarcopenia is a serious global condition encountered in elderly patients, characterized by a disabling course, with its morbidity increasing significantly with advancing age, particularly in individuals over 60 years. In the present study, no significant differences in sarcopenia incidence were observed between male and female patients.

Currently, skeletal muscle is no longer regarded as merely a contractile tissue, but rather as an interface of complex interactions. In addition to the loss of muscle mass and the development of contractile dysfunction, sarcopenia also involves metabolic and endocrine alterations, as well as a state of systemic inflammation commonly present in elderly patients. The process of muscle tissue loss involves a significant decline in protein regeneration, associated with accelerated apoptosis and increased proteolysis (13-15).

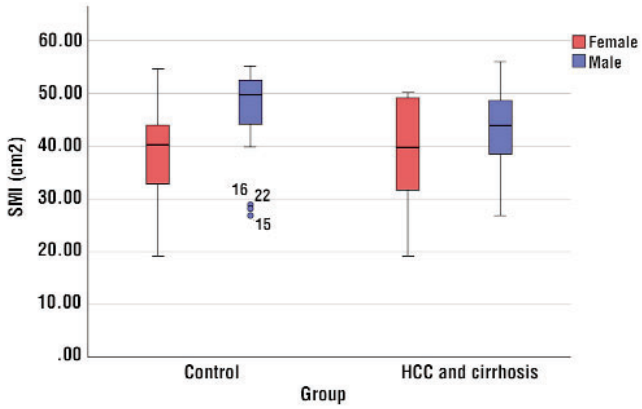

Figure 8. Comparison of SMI evels according to the study groups

In patients with liver cirrhosis, sarcopenia is secondary to the underlying disease, reduced physical activity, and/or malnutrition (protein deficiency) (16,17).

A biomarker has been defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (18). Therefore, a biomarker may represent a clinical tool, a specific molecule, or a biological characteristic that can be detected through medical imaging techniques. Sarcopenia-specific biomarkers may enable the identification of patients who already have this condition or are at risk of developing it, as well as the monitoring of therapeutic and preventive interventions.

Osteonectin, also known as SPARC (secreted protein acidic and rich in cysteine), is an extracellular matrix glycoprotein secreted by most tissues and involved in bone mineralization and cell–matrix interactions (3). Elevated osteonectin levels have been reported in patients with various myopathies, such as

Duchenne muscular dystrophy and congenital muscular dystrophy (19). Increased osteonectin levels in cirrhotic patients are associated with hepatic fibrosis, with studies demonstrating that reduced SPARC expression ameliorates liver fibrosis in vivo in murine models (23).

The C-terminal agrin fragment has been extensively studied as a biomarker of muscle dysfunction, with increased circulating levels representing an early indicator of neuromuscular junction impairment and muscle fiber denervation (24,25). Previous studies have reported elevated circulating CAF levels in sarcopenic patients (26–28), findings that are consistent with our results. It should be noted that CAF levels may also be elevated in other conditions associated with muscle mass loss (such as diabetes mellitus, chronic obstructive pulmonary disease, chronic heart failure, and stroke). However, the literature identifies CAF as one of the key potential biomarkers for differentiating sarcopenic from non-sarcopenic patients (29).

The N-terminal propeptide of type III procollagen is released into circulation during the final stage of collagen biosynthesis (30). Elevated P3NP levels have been observed in abnormal collagen synthesis processes and soft tissue fibrosis (31,32). Given that muscle mass declines with age, increased P3NP levels in elderly individuals may indicate skeletal muscle fibrosis (33). Similar to previous studies (34), elevated plasma P3NP levels were also identified in the sarcopenic patients included in our study.

Several studies have demonstrated increased serum P3NP levels in patients with liver disease (35,36), a finding that was also observed in the cirrhotic patient group in our study.

Myostatin, also known as growth differentiation factor-8 (GDF-8), is a negative regulator of muscle growth and was first described by McPherron et al., who demonstrated that suppression of the myostatin gene in mice leads to a hypermuscular phenotype (37). Myostatin is predominantly expressed in skeletal muscle tissue, although its expression has also been identified in cardiac muscle and adipose tissue (38). Consistent with findings from the literature, myostatin levels were elevated in sarcopenic patients compared to the control group (39).

Beyond their diagnostic relevance, the evaluated biomarkers may also have important clinical applications in routine practice. The combined assessment of osteonectin, CAF, P3NP, and myostatin alongside CT-based muscle evaluation could facilitate earlier identification of patients at increased risk for sarcopenia and progressive functional decline. These biomarkers may serve as complementary non-invasive tools for risk stratification and longitudinal monitoring, particularly in cirrhotic patients undergoing nutritional, physical, or pharmacological interventions. Early recognition of muscle deterioration may support timely implementation of individualized therapeutic strategies and potentially improve clinical outcomes in patients with advanced liver disease.

Although the findings in the literature are contradictory, with studies reporting variable levels in elderly patients (38,40), a recent study suggests a decrease in serum myostatin levels with the progression of liver cirrhosis and associates this finding with decompensation and increased mortality (41). Even though myostatin does not appear to be a valid standalone biomarker for sarcopenia, its measurement should be considered in combination with other biomarkers.

Only ≤1 mL of plasma is required for biomarker assessment, and this small volume can be obtained during routine blood tests, thereby reducing patient discomfort and the need for multiple venipunctures. Biomarker measurement and analysis can be completed within a few hours, providing timely information for rapid clinical decision-making.

The analysis of multiple biomarkers for the diagnosis of sarcopenia should not be limited to the four examples used in this study. Further investigations are needed to identify optimal combinations with novel biomarkers that more accurately reflect the entire biological cascade associated with sarcopenia.

Our study has several limitations. First, it is a single-center study with a relatively small sample size. Larger prospective studies are required to confirm the association between serum biomarker levels and sarcopenia in liver cirrhosis, as the influence of undiagnosed conditions at the time of evaluation cannot be excluded. Additionally, certain factors such as physical activity, cognitive status, nutrition, and medication were not included in the analysis due to lack of available data, although they may influence the variability of serum biomarker levels. The relatively small sample size represents an important limitation of the present study and may reduce the overall statistical power and generalizability of the findings. Consequently, larger prospective multicenter studies involving more heterogeneous patient populations are required to validate the observed associations and further clarify the clinical utility of these biomarkers in cirrhosis-associated sarcopenia.

Also, the inclusion of patients with liver cirrhosis both with and without hepatocellular carcinoma may have introduced a degree of heterogeneity within the study cohort, potentially influencing biomarker profiles and the interpretation of the results. This

aspect was considered during data interpretation and is acknowledged as a study limitation.

Although multivariate analyses could have provided additional adjustment for potential confounding factors, the relatively small sample size and the uneven distribution of certain clinical variables limited the feasibility of constructing statistically robust multivariable models without a substantial risk of overfitting.

Conclusions

Sarcopenia frequently accompanies advanced liver disease, is rarely reversible, and represents a negative prognostic factor. The pathogenic mechanisms underlying sarcopenia in patients with liver cirrhosis are complex and include malnutrition, anabolic resistance, and metabolic imbalances (hyperammonemia, hormonal instability, and chronic inflammation). Serum biomarkers provide a convenient, non-invasive, and rapid method for assessing the risk of sarcopenia, making them an excellent option for screening at-risk populations.

Our study identified higher levels of CAF, P3NP, osteonectin, and myostatin in sarcopenic patients compared to the control group. It is important to emphasize that further studies on much larger cohorts are essential to explore and clarify the role of biomarkers in the molecular mechanisms underlying sarcopenia associated with liver cirrhosis.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Statement

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Sf. Apostol Andrei County Emergency Clinical Hospital, Constanța, Romania (Approval No. 05/04.02.2022). Written informed consent was obtained from all participants prior to enrollment in the study.

References

1. Roubenoff R. Sarcopenia: a major modifiable cause of frailty in the elderly. J Nutr Health Aging. 2000;4(3):140-2.
2. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019;48(1): 16-31.
3. Kwak JY, Hwang H, Kim S-K, Choi JY, Lee S-M, Bang H, et al Prediction of sarcopenia using a combination of multiple serum biomarkers. Sci Rep. 2018;8(1):8574.
4. Scharf G, Heineke J, Finding good biomarkers for sarcopenia. J Cachexia Sarcopenia Muscle. 2012;3(3):145-8.
5. Fox R, Stenning K, Slee A, Macnaughtan J, Davies N, et al. Sarcopenia in liver cirrhosis: Prevalence, pathophysiology and therapeutic strategies. Anal Biochem. 2022;647:114581.
6. Tagliafico AS, Bignotti B, Torri L, Rossi F. Sarcopenia: how to measure, when and why. Radiol Med. 2022;127(3):228-237.
7. Boutin RD, Kaptuch JM, Bateni CP, Chalfant JS, Yao L. Influence of IV Contrast Administration on CT Measures of Muscle and Bone Attenuation: Implications for Sarcopenia and Osteoporosis Evaluation. AJR Am J Roentgenol. 2016;207(5): 1046-1054.
8. Amini B, Boyle SP, Boutin RD, Lenchik L. Approaches to Assessment of Muscle Mass and Myosteatosis on Computed Tomography: A Systematic Review. J Gerontol A Biol Sci Med Sci. 2019;74(10):1671-1678.
9. Marasco G, Serenari M, Renzulli M, Alemanni LV, Rossini B, Pettinari I, et al. Clinical impact of sarcopenia assessment in patients with hepatocellular carcinoma undergoing treatments. J Gastroenterol. 2020;55(10):927-943.
10. Locquet M, Bruyère O, Lengelé L, Reginster JY, Beaudart C. Relationship between smoking and the incidence of sarcopenia: The SarcoPhAge cohort. Public Health. 2021;193:101-108.
11. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigaré R, et al (2014) An Official American Thoracic Society/European Respiratory Society Statement: Update on Limb Muscle Dysfunction in Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2014;189(9):e15-62.
12. Pechmann LM, Jonasson TH, Canossa VS, Trierweiler H, Kisielewicz G, Petterle RR, et al. Sarcopenia in Type 2 Diabetes Mellitus: A Cross-Sectional Observational Study. Int J Endocrinol. 2020;2020:7841390.
13. Pascual-Fernández J, Fernández-Montero A, Córdova-Martínez A, Pastor JD, Martínez-Rodríguez A, Roche E. Sarcopenia: Molecular Pathways and Potential Targets for Intervention. Int J Mol Sci. 2020;21(22):8844.
14. Kalinkovich A, Livshits G. Sarcopenic obesity or obese sarcopenia: A cross talk between age-associated adipose tissue and skeletal muscle inflammation as a main mechanism of the pathogenesis. Ageing Res Rev. 2017;35:200-221.
15. Tournadre A, Vial G, Capel F, Soubrier M, Boirie Y. Sarcopenia. Joint Bone Spine. 2019;86(3):309-314.
16. Anand AC. Nutrition and Muscle in Cirrhosis. J Clin Exp Hepatol. 2017;7(4):340-357.
17. Romiti A, Merli M, Martorano M, Parrilli G, Martino F, Riggio O, et al. Malabsorption and nutritional abnormalities in patients with liver cirrhosis. Ital J Gastroenterol. 1990;22(3):118-23.
18. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89-95.
19. Jørgensen LH, Petersson SJ, Sellathurai J, Andersen DC, Thayssen S, Sant DJ, et al. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. J Histochem Cytochem. 2009;57(1):29-39.
20. Nakamura K, Nakano S-I, Miyoshi T, Yamanouchi K, Nishihara M. Loss of sparc in mouse skeletal muscle causes myofiber atrophy. Muscle Nerve. 2013;48(5):791-9.
21. Nakamura K, Yamanouchi K, Nishihara M. Transdisciplinary Approach for Sarcopenia. Molecular mechanism of sarcopenia: The role of skeletal muscle niche component SPARC in the regulation of myogenesis and adipogenesis and its alteration with age). Clin Calcium. 2014 Oct;24(10):1471-8. Japanese
22. Akutsu T, Ito E, Narita M, Ohdaira H, Suzuki Y, Urashima M. Effect of Serum SPARC Levels on Survival in Patients with Digestive Tract Cancer: A Post Hoc Analysis of the AMATERASU Randomized Clinical Trial. Cancers (Basel). 2020;12(6):1465.
23. Atorrasagasti C, Aquino JB, Hofman L, Alaniz L, Malvicini M, Garcia M, et al. SPARC downregulation attenuates the profibrogenic response of hepatic stellate cells induced by TGF-β1 and PDGF. Am J Physiol Gastrointest Liver Physiol. 2011;300(5):G739-48.
24. Pratt J, De Vito G, Narici M, Segurado R, Pessanha L, Dolan J, et al. Plasma C-Terminal Agrin Fragment as an Early Biomarker for Sarcopenia: Results From the GenoFit Study. J Gerontol A Biol Sci Med Sci. 2021;76(12):2090-2096.
25. Hepple RT, Rice CL. Innervation and neuromuscular control in ageing skeletal muscle. J Physiol. 2016;594(8):1965-78.
26. Marzetti E, Calvani R, Lorenzi M, Marini F, D'Angelo E, Martone AM, et al. Serum levels of C-terminal agrin fragment (CAF) are associated with sarcopenia in older hip fractured patients. Exp Gerontol. 2014;60:79-82.
27. Hettwer S, Dahinden P, Kucsera S, Farina C, Ahmed S, Fariello R, et al. Elevated levels of a C-terminal agrin fragment identifies a new subset of sarcopenia patients. Exp Gerontol. 2013;48(1):69-75.
28. Landi F, Calvani R, Lorenzi M, Martone AM, Tosato M, Drey M, et al. Serum levels of C-terminal agrin fragment (CAF) are associated with sarcopenia in older multimorbid

community-dwellers: Results from the ilSIRENTE study. Exp Gerontol. 2016;79:31-6.
29. Monti E, Sarto F, Sartori R, Zanchettin G, Löfler S, Kern H, et al. C-terminal agrin fragment as a biomarker of muscle wasting and weakness: a narrative review. J Cachexia Sarcopenia Muscle. 2023;14(2):730-744.
30. Prockop DJ, Kivirikko KI, Tuderman L, Guzman NA. The biosynthesis of collagen and its disorders (first of two parts). N Engl J Med. 1979;301(1):13-23.
31. Agarwal I, Glazer NL, Barasch E, Biggs ML, Djoussé L, Fitzpatrick AL, et al. Fibrosis-related biomarkers and risk of total and cause-specific mortality: the cardiovascular health study. Am J Epidemiol. 2014;179(11):1331-9.
32. Morine KJ, Paruchuri V, Qiao X, Mohammad N, Mcgraw A, Yunis A, et al. Circulating multimarker profile of patients with symptomatic heart failure supports enhanced fibrotic degradation and decreased angiogenesis. Biomarkers. 2016;21(1):91-7.
33. Shin HE, Kim M, Won CW. Association between plasma procollagen type III N-terminal peptide (P3NP) levels and physical performance in elderly men: The Korean Frailty and Aging Cohort Study (KFACS). Exp Gerontol. 2021;154:111523.
34. Qaisar R, Karim A, Muhammad T, Shah I, Khan J. Prediction of sarcopenia using a battery of circulating biomarkers. Sci Rep. 2021;11(1):8632.
35. Gudowska M, Gruszewska E, Panasiuk A, Cylwik B, Swiderska M, Flisiak R, et al. High serum N-terminal propeptide of procollagen type III concentration is associated with liver diseases. Prz Gastroenterol. 2017;12(3):203-207.
36. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest. 2005;115(2):209-18.
37. McPherron AC, Lawler AM, Lee S-J. Regulation of skeletal muscle mass in mice by a new TGF-p superfamily member. Nature. 1997;387(6628):83-90.
38. Baczek J, Silkiewicz M, Wojszel ZB. Myostatin as a Biomarker of Muscle Wasting and other Pathologies-State of the Art and Knowledge Gaps. Nutrients. 2020;12(8):2401.
39. Dasarathy S. Myostatin and beyond in cirrhosis: all roads lead to sarcopenia. J Cachexia Sarcopenia Muscle. 2017;8(6):864-869.
40. Peng L, Lee W, Liu L, Lin M-H, Chen L-K. Healthy community-living older men differ from women in associations between myostatin levels and skeletal muscle mass. J Cachexia Sarcopenia Muscle. 2018;9(4):635-642.
41. Ruiz-Margáin A, Pohlmann A, Lanzerath S, Langheinrich M, Campos-Murguía A, Román-Calleja BM, et al (2023) Myostatin is associated with the presence and development of acute-on-chronic liver failure. JHEP Rep. 2023;5(8):100761.