

Elevated serum myostatin level is associated with worse survival in patients with liver cirrhosis

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Abstract

Background We aimed to elucidate the relationship between serum myostatin levels and other markers including skeletal muscle mass and to investigate the influence of serum myostatin levels on survival for patients with liver cirrhosis (LC).

Methods A total of 198 LC subjects were analysed in this study. Myostatin levels were measured using stored sera. We retrospectively investigated the relationship between myostatin level and other markers, and the influence of myostatin level on overall survival (OS). Assessment of skeletal muscle mass was performed using the psoas muscle index (PMI) on computed tomography images at baseline. PMI indicates the sum of bilateral psoas muscle mass calculated by hand tracing at the lumbar three level on computed tomography images divided by height squared (cm^2/m^2). The study cohort was divided into two groups based on the median myostatin value in each gender.

Results Our study cohort included 108 male and 90 female patients with a median age of 67.5 years. The median (range) myostatin level for male patients was 3419.6 pg/mL (578.4–12897.7 pg/mL), whereas that for female patients was 2662.4 pg/mL (710.4–8782.0 pg/mL) ($P = 0.0024$). Median (range) serum myostatin level for Child–Pugh A patients ($n = 123$) was 2726.0 pg/mL (578.4–12667.2 pg/mL), whereas that for Child–Pugh B or C patients ($n = 75$) was 3615.2 pg/mL (663.3–12897.7 pg/mL) ($P = 0.0011$). For the entire cohort, the 1-, 3-, 5-, and 7-year cumulative OS rates were 93.94%, 72.71%, 50.37%, and 38.47%, respectively, in the high-myostatin group and 96.97%, 83.27%, 73.60%, and 69.95%, respectively, in the low-myostatin group ($P = 0.0001$). After excluding hepatocellular carcinoma patients (at baseline) from our analysis ($n = 158$), the 1-, 3-, 5-, and 7-year cumulative OS rates were 96.0%, 77.93%, 52.97%, and 39.08%, respectively, in the high-myostatin group and 96.39%, 87.58%, 77.63%, and 73.24%, respectively, in the low-myostatin group ($P = 0.0005$). Higher age ($P = 0.0111$) and lower PMI ($P < 0.0001$) were identified as significant predictors of poorer OS in our multivariate analysis, while higher serum myostatin ($P = 0.0855$) tended to be a significant adverse predictor. In both genders, PMI, serum albumin, prothrombin time, and branched-chain amino acid to tyrosine ratio showed a significantly inverse correlation with myostatin levels, and serum ammonia levels showed a significantly positive correlation with myostatin levels.

Conclusions Higher serum myostatin levels correlated with muscle mass loss, hyperammonemia, and impaired protein synthesis, as reflected by lower serum albumin levels and lower branched-chain amino acid to tyrosine ratio levels. High serum myostatin levels were also associated with a reduced OS rate in LC patients.

Keywords Liver cirrhosis; Serum myostatin; Skeletal muscle; Predictor; Prognosis

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Introduction

Sarcopenia is a clinical disease entity defined by skeletal muscle mass depletion and muscle strength weakness. This disease has received significant attention from clinicians recently because of its significant deleterious influence on outcomes.^{1–10} Generally, changes in skeletal muscle mass can occur with increasing age, and muscle mass depletion is a common disorder in the ageing population.¹¹ Liver cirrhosis (LC) results from the progression of multiple forms of necro-inflammatory disorders of the liver by fibrosis and vascular remodelling and can be related to secondary sarcopenia because of two metabolic burdens in LC patients: protein metabolic disorder and energy metabolic disorder.^{2,5,6,12–14} Sarcopenia is also linked to the prognosis of patients with liver diseases.^{15–20} Skeletal muscle mass is maintained by the balance between protein synthesis and protein breakdown, and sarcopenia can occur because of an increase in proteolysis or a reduction in protein synthesis or both.^{1–10}

Myostatin is a cytokine belonging to the transforming growth factor beta family, and its functional role was first elucidated in 1997.²¹ Myostatin is a negative regulator of muscle protein synthesis, which acts via the impaired mammalian target of rapamycin signalling, which strongly suppresses skeletal muscle growth.²² Previous studies have reported an increased skeletal muscle mass in mice given a molecule that inhibits myostatin.²¹ In addition, the serum myostatin concentrations of patients with LC have been reported to be significantly higher than those of healthy controls, thus indicating that myostatin is associated with the loss of skeletal muscle mass loss.^{22,23} A previous study also determined that elevated ammonia levels in the blood lead to increased myostatin expression in the skeletal muscle.²⁴ Myostatin can be transcriptionally up-regulated by hyperammonemia in the skeletal muscle of humans.^{10,22,25,26} An excessive alcohol intake has also been demonstrated to negatively impact protein metabolism and can lead to the elevation of myostatin expression in skeletal muscle.^{8,27} Myostatin deficiency has more recently been demonstrated to exert beneficial effects on metabolism, adiposity, and insulin sensitivity.^{28,29} On the other hand, follistatin is a liver-derived inhibitor of myostatin, and a recent study has reported that follistatin levels in patients with LC are significantly reduced as compared with healthy controls, which might contribute to the loss of muscle mass in LC patients.³⁰

However, whether serum myostatin levels are associated with clinical outcomes for patients with LC remains an unsolved issue. Addressing these clinical questions may be of importance because of the lack of relevant clinical data. The aims of this present study were thus to elucidate the relationship between serum myostatin levels and other markers including skeletal muscle mass and to investigate

the influence of serum myostatin levels on survival for patients with LC.

Patients and methods

Patients

Between February 2006 and January 2015, a total of 213 LC individuals, who had stored sera available for the measurement of myostatin level, were admitted at the Division of Hepatobiliary and Pancreatic disease, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan. In our department, stored serum samples are generally obtained from all hospitalized patients in order that their nutritional status can be assessed, once informed written consent has been obtained. Serum myostatin was measured using commercially available kits (R&D Systems, Minneapolis, MN, USA). Assessment of skeletal muscle mass was performed using the psoas muscle index (PMI) on computed tomography (CT) images at baseline. PMI indicates the sum of bilateral psoas muscle mass calculated by hand tracing at the lumbar three (L3) level on CT images divided by height squared (cm^2/m^2).³¹ A lower PMI was defined as $<6.36 \text{ cm}^2/\text{m}^2$ for male patients and $<3.92 \text{ cm}^2/\text{m}^2$ for female patients according to the recommendations of current guidelines.⁵ In total, 213 subjects had available data for myostatin level, PMI at baseline, and clinical outcomes. Of these patients, advanced hepatocellular carcinoma (HCC) patients at baseline in whom curative therapies were not possible ($n = 15$) were excluded from the current analysis because advanced malignancies themselves can affect skeletal muscle mass.^{32,33} A total of 198 LC subjects were therefore analysed in this study. Follow-up observation included periodical blood tests and radiological assessments by ultrasonography, CT, or magnetic resonance imaging in order to detect HCC incidence or recurrence every 3–6 months. None of the patients received liver transplantation during the follow-up period. LC was determined by pathological findings, radiological findings such as ultrasonography, CT, or magnetic resonance imaging, and/or laboratory data including markers of liver fibrosis.^{34–36} In principal, for patients with a lower serum albumin level (less than 3.5 g/dL), liver supporting therapies, including branched-chain amino acid (BCAA) treatment or late evening meals with BCAA-enriched snacks, were performed.^{37–39} For patients with hepatitis virus-related LC, antiviral therapies, including direct acting antivirals, interferon-based regimens, or nucleoside analogue therapy, were also considered.^{37,38} Alcoholic LC patients were instructed to cease all alcohol intake. For patients with other liver disease etiologies, the most appropriate therapy for each underlying liver disease was carried out. We retrospectively investigated the relationship

between serum myostatin level and other markers, and the influence of serum myostatin level on survival. Factors linked to overall survival (OS) in the univariate and multivariate analyses were also examined. Diagnosis and treatment choices for HCC were as previously described.^{40,41}

The ethical committee of Hyogo College of Medicine approved the current study protocol, and this study strictly followed all regulations of the Declaration of Helsinki.

Statistical analyses

Quantitative variables were compared by unpaired *t*-test, Mann–Whitney *U* test, or Spearman's rank correlation coefficient r_s as appropriate. Continuous variables were divided into two groups using median values that were then treated as dichotomous covariates for univariate analysis except for age, body mass index, and PMI. The cut-off values for age and body mass index for univariate analysis were 65 years and 25.0 kg/m². As described earlier, cut-off values for PMI were set at 6.36 cm²/m² for male patients and 3.92 cm²/m² for female patients. Survival curves were created using the Kaplan–Meier method and compared by the log-rank test. Variables with a *P* value <0.05 in our univariate analysis were finally entered into the multivariate analysis using the Cox proportional hazard model. OS was defined as the duration from the blood collection date for stored sera for measuring myostatin level until death from any cause or the last follow-up visit.

Data are presented as the median value (range) unless otherwise stated. *P* values of less than 0.05 were considered to indicate statistical significance. Statistical analysis was performed with JMP 11 software (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics

The baseline characteristics in this study (*n* = 198) are shown in Table 1. The study included 108 male and 90 female patients with a median age (range) of 67.5 (22.6–89.8) years. At baseline, HCC was identified on radiological findings in 40 patients (20.2%) and ascites in 35 patients (17.7%). Patients were predominantly hepatitis C virus (HCV)-related LC (116/198, 58.6%). Child–Pugh A was in the majority (123/198, 62.1%). The median follow-up period was 4.35 years (range: 0.19–10.48 years), while the median (range) serum myostatin level for the entire cohort was 2994.4 pg/mL (578.4–12897.7 pg/mL). The median (range) serum myostatin level for men was 3419.6 pg/mL (578.4–12897.7 pg/mL), whereas that for women was 2662.4 pg/mL (710.4–8782.0 pg/mL)

Table 1 Baseline data (*n* = 198)

Variables	Number or median (range)
Age (years)	67.5 (22.6–89.8)
Gender, male/female	108/90
Body mass index (kg/m ²)	22.77 (13.05–36.61)
Serum myostatin (pg/mL), men	3419.6 (578.4–12897.7)
Serum myostatin (pg/mL), women	2662.4 (710.4–8782.0)
Psoas muscle index (cm ² /m ²), men	5.33 (2.06–9.68)
Psoas muscle index (cm ² /m ²), women	4.02 (0.79–6.70)
Causes of liver disease	17/116/19/46
HBV/HCV/alcoholic/others	
Child–Pugh classification, A/B/C	123/72/3
Presence of HCC, yes/no	40/158
Total bilirubin (mg/dL)	1.1 (0.3–6.0)
Serum albumin (g/dL)	3.6 (2.0–4.9)
Prothrombin time (%)	74.7 (35.6–115.6)
Platelets (×10 ⁴ /mm ³)	8.6 (2.5–42.4)
Serum sodium (mmol/L)	140 (127–145)
Serum creatinine (mg/dL)	0.70 (0.34–4.14)
Total cholesterol (mg/dL)	150 (70–306)
AST (IU/L)	44 (16–402)
ALT (IU/L)	34 (9–487)
Serum ammonia (μg/dL)	40 (7–132)
HOMA-IR	2.51 (0.07–36.41)
BTR	4.03 (1.46–9.67)
Ascites, yes/no	35/163
Alpha-fetoprotein (ng/mL)	5.5 (0.8–1345.0)

Data are expressed as number or median (range). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid to tyrosine ratio; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment of insulin resistance.

(*P* = 0.0024; Figure 1A). The median (range) serum myostatin level for Child–Pugh A patients was 2726.0 pg/mL (578.4–12 667.2 pg/mL), whereas that for Child–Pugh B or C patients was 3615.2 pg/mL (663.3–12897.7 pg/mL) (*P* = 0.0011; Figure 1B). The median (range) PMI for men was 5.33 cm²/m² (2.06–9.68 cm²/m²), whereas that for women was 4.02 cm²/m² (0.79–6.70 cm²/m²) (*P* < 0.0001).

Relationship between serum myostatin level and baseline characteristics for male (*n* = 108) and female (*n* = 90) patients

In male patients, PMI ($r_s = -0.4439$, *P* < 0.0001), serum albumin ($r_s = -0.3844$, *P* < 0.0001), prothrombin time (PT) ($r_s = -0.2279$, *P* = 0.0188), serum ammonia ($r_s = 0.5856$, *P* < 0.0001), and BCAA to tyrosine ratio (BTR) ($r_s = -0.4610$, *P* < 0.0001) were significantly correlated with baseline myostatin level. In female patients, age ($r_s = -0.2232$, *P* = 0.0345), PMI ($r_s = -0.2624$, *P* = 0.0108), total bilirubin ($r_s = -0.2915$, *P* = 0.0053), serum albumin ($r_s = -0.3945$, *P* = 0.0001), PT ($r_s = -0.2537$, *P* = 0.0158), serum ammonia ($r_s = 0.3922$, *P* = 0.0001), and BTR ($r_s = -0.4115$, *P* < 0.0001) were also significantly correlated with baseline myostatin level (Table 2).

Figure 1 (A) Baseline serum myostatin levels in male and female patients. The median (range) level of serum myostatin for male patients was 3419.6 pg/mL (578.4–12897.7 pg/mL) whereas that for females was 2662.4 pg/mL (710.4–8782.0 pg/mL) ($P = 0.0024$). (B) Baseline serum myostatin levels according to Child–Pugh classification. The median (range) serum myostatin level for Child–Pugh A patients was 2726.0 pg/mL (578.4–12667.2 pg/mL) whereas that for Child–Pugh B or C was 3615.2 pg/mL (663.3–12897.7 pg/mL) ($P = 0.0011$).

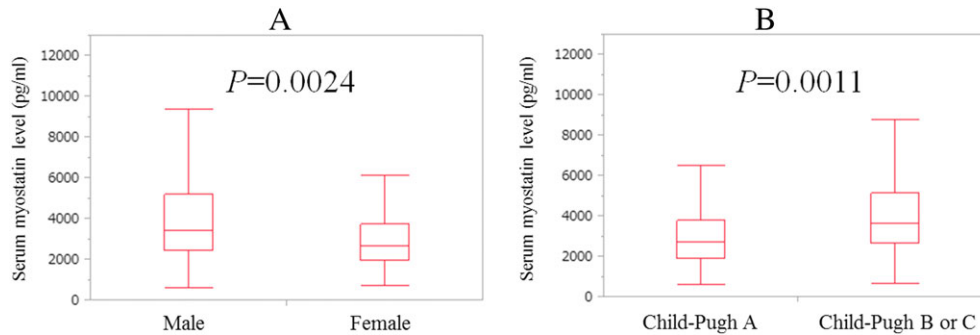


Table 2 Relationship between serum myostatin level and baseline characteristics for male ($n = 108$) and female ($n = 90$) patients

	Male		Female	
	r_s	P value	r_s	P value
Age	0.1881	0.0513	−0.2232	0.0345
Body mass index	−0.0751	0.4398	0.1126	0.2908
Psoas muscle index	−0.4439	<0.0001	−0.2624	0.0108
Total bilirubin	0.0725	0.4558	0.2915	0.0053
Serum albumin	−0.3844	<0.0001	−0.3945	0.0001
Prothrombin time	−0.2279	0.0188	−0.2537	0.0158
Platelets	−0.0871	0.3702	−0.1140	0.2848
Serum sodium	−0.0156	0.8923	0.1383	0.1934
Serum creatinine	−0.0568	0.5591	−0.1403	0.1873
Total cholesterol	−0.0239	0.8062	−0.1099	0.3026
AST	0.1593	0.0996	0.0829	0.2499
ALT	0.1240	0.2009	0.0885	0.4070
Serum ammonia	0.5856	<0.0001	0.3922	0.0001
HOMA-IR	−0.1069	0.2708	0.1507	0.1661
BTR	−0.4610	<0.0001	−0.4115	<0.0001
Alpha-fetoprotein	0.1408	0.1461	0.1159	0.2767

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid to tyrosine ratio; HOMA-IR, homeostasis model assessment of insulin resistance.

Cumulative overall survival rates for the entire cohort according to serum myostatin level

Baseline serum myostatin levels were significantly different when compared between male and female patients ($P = 0.0024$). We thus defined patients with a serum myostatin level, which was higher than the median value for each gender as the high-myostatin group ($n = 99$) and those with less than the median value for each gender as the low-myostatin group ($n = 99$).

For the entire cohort, the 1-, 3-, 5-, and 7-year cumulative OS rates were 93.94%, 72.71%, 50.37%, and 38.47%, respectively, in the high-myostatin group and 96.97%, 83.27%, 73.60%, and 69.95%, respectively, in the low-myostatin group ($P = 0.0001$; Figure 2A). After excluding HCC

patients (at baseline) from our analysis ($n = 158$), the 1-, 3-, 5-, and 7-year cumulative OS rates were 96.0%, 77.93%, 52.97%, and 39.08%, respectively, in the high-myostatin group and 96.39%, 87.58%, 77.63%, and 73.24%, respectively, in the low-myostatin group ($P = 0.0005$; Figure 2B).

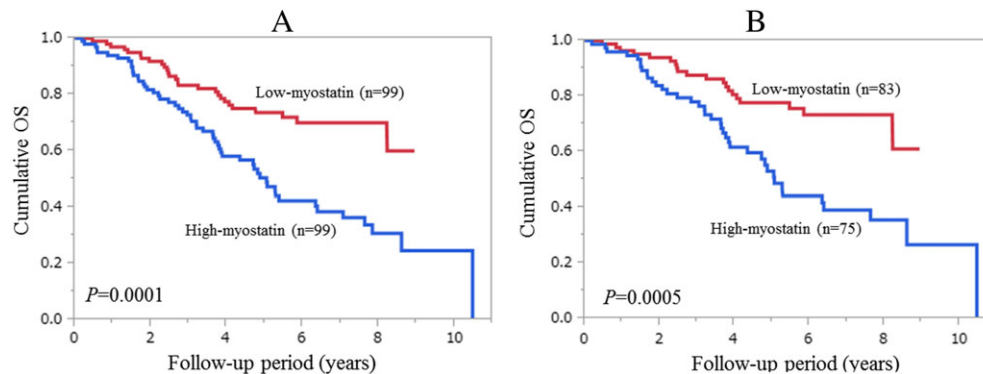
Causes of death for male and female patients

In the high-myostatin group, 55 patients (55.6%) died during the follow-up period. The causes of death were liver failure in 34 patients, HCC progression in 15 patients, and miscellaneous causes in six patients. In the low-myostatin group, 27 patients (27.3%) died during the follow-up period. The causes of death were liver failure in 11 patients, HCC progression in 14 patients, and miscellaneous causes in two patients.

Univariate and multivariate analyses of parameters contributing to overall survival

Univariate analysis identified the following factors as being significantly associated with a worse OS: age ≥ 65 years ($P = 0.0247$); higher serum myostatin level ($P = 0.0001$); lower PMI ($P < 0.0001$); presence of HCC ($P = 0.0135$); PT $< 74.7\%$ ($P = 0.0189$); serum ammonia ≥ 40 $\mu\text{g/dL}$ ($P = 0.0252$); alpha-fetoprotein (AFP) ≥ 5.5 ng/mL ($P = 0.0117$); presence of ascites ($P < 0.0001$); and a higher Child–Pugh score ($P = 0.0289$; Table 3). Because the Child–Pugh score includes PT and ascites, these parameters were not included in our multivariate analysis. To avoid the influence of collinearity between AFP and the presence of HCC, AFP was not included in the multivariate analysis. The hazard ratios and 95% confidence intervals calculated by multivariate analysis for the six significant parameters ($P < 0.05$) in the univariate analysis are presented in Table 3.

Figure 2 (A) Cumulative overall survival (OS) rates for the entire cohort ($n = 198$). The 1-, 3-, 5-, and 7-year cumulative OS rates were 93.94%, 72.71%, 50.37%, and 38.47%, respectively, in the high-myostatin group and 96.97%, 83.27%, 73.60%, and 69.95%, respectively, in the low-myostatin group ($P = 0.0001$). (B) Cumulative OS rates after excluding hepatocellular carcinoma patients at baseline ($n = 158$). The 1-, 3-, 5-, and 7-year cumulative OS rates were 96.0%, 77.93%, 52.97%, and 39.08%, respectively, in the high-myostatin group and 96.39%, 87.58%, 77.63%, and 73.24%, respectively, in the low-myostatin group ($P = 0.0005$).



Univariate and multivariate analyses of parameters contributing to overall survival after excluding hepatocellular carcinoma patients at baseline ($n = 158$)

We also carried out additional analyses after excluding HCC patients at baseline. Univariate analysis identified the following factors as being significantly associated with a worse OS: age ≥ 65 years ($P = 0.0385$); higher serum myostatin level ($P = 0.0005$); lower PMI ($P < 0.0001$); alanine aminotransferase ≥ 32 IU/L ($P = 0.0201$); serum albumin ≥ 3.6 g/dL ($P = 0.0105$); PT $< 74.7\%$ ($P = 0.0233$); serum ammonia ≥ 39.5 μ g/dL ($P = 0.0220$); BTR < 4.04 ($P = 0.0276$); presence of ascites ($P < 0.0001$); and a higher Child–Pugh score ($P = 0.0065$; Table 4). Because the Child–Pugh score includes albumin, PT, and ascites, these parameters were not included in our multivariate analysis. The hazard ratios and 95% confidence intervals calculated by multivariate analysis for the seven significant parameters ($P < 0.05$) in the univariate analysis are presented in Table 4.

Comparison of baseline characteristics between the high-myostatin group and the low-myostatin group

Data for comparison of baseline characteristics between the high-myostatin group and the low-myostatin group are presented in Table 5. Significant differences were detected between the two groups in terms of PMI for male patients ($P < 0.0001$), PMI for female patients ($P = 0.0175$), Child–Pugh classification ($P = 0.0206$), serum albumin ($P < 0.0001$), PT ($P = 0.0147$), serum ammonia ($P < 0.0001$), BTR ($P < 0.0001$), and AFP ($P = 0.0422$).

The effect of serum myostatin on survival according to age

In patients with a higher age (65 years or more, $n = 112$), patients in the low-myostatin group ($n = 51$) had a significantly higher survival rate than those in the high-myostatin group ($n = 61$) ($P = 0.0041$; Figure 3A). Similarly, in patients with a lower age (less than 65 years, $n = 86$), patients in the low-myostatin group ($n = 48$) had a significantly higher survival rate than those in the high-myostatin group ($n = 38$) ($P = 0.0193$; Figure 3B).

The effect of serum myostatin on survival according to gender

In men, patients in the low-myostatin group ($n = 54$) had a significantly higher survival rate than those in the high-myostatin group ($n = 54$) ($P = 0.0040$; Figure 3C). In the same way, in women, patients in the low-myostatin group ($n = 45$) had a significantly higher survival rate than those in the high-myostatin group ($n = 45$) ($P = 0.0110$; Figure 3D).

The effect of serum myostatin on survival according to Child–Pugh classification

In patients with Child–Pugh A ($n = 123$), patients in the low-myostatin group ($n = 71$) had a significantly higher survival rate than those in the high-myostatin group ($n = 52$) ($P = 0.0011$; Figure 4A). In patients with Child–Pugh B or C ($n = 75$), patients in the low-myostatin group ($n = 28$) did not have a significantly higher survival rate than those in the high-myostatin group ($n = 47$) ($P = 0.1116$; Figure 4B).

Table 3 Univariate and multivariate analyses of factors linked to overall survival ($n = 198$)

Variables	Number of each category	Univariate
		P value
Age (years) ≥ 65 , yes/no	112/86	0.0247
Gender, male/female	108/90	0.1907
Cause of liver diseases, B/C/alcoholic/others	17/116/19/46	0.3955
Serum myostatin, high/low	99/99	0.0001
Psoas muscle index, high/low	90/108	<0.0001
Presence of HCC, yes/no	40/158	0.0135
AST ≥ 44 IU/L, yes/no	100/98	0.9389
ALT ≥ 34 IU/L, yes/no	100/98	0.9857
Serum albumin ≥ 3.6 g/dL, yes/no	104/94	0.0615
Total bilirubin ≥ 1.1 mg/dL, yes/no	99/99	0.7636
Prothrombin time $\geq 74.7\%$, yes/no	100/98	0.0189
Platelet count $\geq 8.6 \times 10^4/\text{mm}^3$, yes/no	99/99	0.0551
Serum creatinine ≥ 0.7 mg/dL, yes/no	102/96	0.3043
Total cholesterol ≥ 150 mg/dL, yes/no	103/95	0.3741
Serum sodium ≥ 140 mmol/L, yes/no	100/98	0.0635
Serum ammonia ≥ 40 $\mu\text{g}/\text{dL}$, yes/no	102/96	0.0252
HOMA-IR ≥ 2.51 , yes/no	100/98	0.1589
BTR ≥ 4.03 , yes/no	101/97	0.1138
Alpha-fetoprotein ≥ 5.5 ng/mL, yes/no	99/99	0.0117
Body mass index ≥ 25.0 kg/m ² , yes/no	56/142	0.0761
Ascites, yes/no	35/163	<0.0001
Child-Pugh, A/B or C	123/75	0.0289

Variables	Multivariate analysis		
	Hazard ratio	95% confidence interval	P value
Age (per 1 year)	1.030	1.007–1.057	0.0111
Serum myostatin, high/low	1.576	0.936–2.574	0.0855
Psoas muscle index, high/low	0.229	0.124–0.394	<0.0001
Presence of HCC	1.475	0.899–2.468	0.1246
Serum ammonia (per 1 $\mu\text{g}/\text{dL}$)	1.008	0.996–1.019	0.1976
Child-Pugh A/B or C	0.716	0.451–1.142	0.1590

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid to tyrosine ratio; HCC, hepatocellular carcinoma; HOMA-IR, homeostasis model assessment of insulin resistance.

The effect of serum myostatin on survival according to psoas muscle index

In patients with a higher PMI (greater than the cut-off PMI value in each gender (men, 6.36 cm²/m²; women, 3.92 cm²/m²), $n = 90$), patients in the low-myostatin group ($n = 57$) had a significantly higher survival rate than those in

Table 4 Univariate and multivariate analyses of factors linked to overall survival after excluding HCC patients at baseline ($n = 158$)

Variables	Number of each category	Univariate
		P value
Age (years) ≥ 65 , yes/no	84/74	0.0385
Gender, male/female	90/68	0.2192
Cause of liver diseases, B/C/alcoholic/others	16/81/17/44	0.3889
Serum myostatin, high/low	75/83	0.0005
Psoas muscle index, high/low	84/74	<0.0001
AST ≥ 40.5 IU/L, yes/no	79/79	0.9411
ALT ≥ 32 IU/L, yes/no	80/78	0.0201
Serum albumin ≥ 3.6 g/dL, yes/no	84/74	0.0105
Total bilirubin ≥ 1.1 mg/dL, yes/no	85/73	0.1987
Prothrombin time $\geq 74.7\%$, yes/no	80/78	0.0233
Platelet count $\geq 8.4 \times 10^4/\text{mm}^3$, yes/no	79/79	0.0527
Serum creatinine ≥ 0.71 mg/dL, yes/no	80/78	0.1083
Total cholesterol ≥ 151 mg/dL, yes/no	80/78	0.3039
Serum sodium ≥ 140 mmol/L, yes/no	82/76	0.1847
Serum ammonia ≥ 39.5 $\mu\text{g}/\text{dL}$, yes/no	79/79	0.0220
HOMA-IR ≥ 2.55 , yes/no	79/79	0.1846
BTR ≥ 4.04 , yes/no	79/79	0.0276
Alpha-fetoprotein ≥ 4.5 ng/mL, yes/no	81/77	0.1886
Body mass index ≥ 25.0 kg/m ² , yes/no	43/115	0.2106
Ascites, yes/no	33/125	<0.0001
Child-Pugh, A/B or C	96/62	0.0065

Variables	Multivariate analysis		
	Hazard ratio	95% confidence interval	P value
Age (per 1 year)	1.026	0.998–1.056	0.0733
Serum myostatin, high/low	1.717	0.942–3.215	0.0778
Psoas muscle index, high/low	0.229	0.108–0.442	<0.0001
ALT (per 1 IU/L)	1.015	1.002–1.031	0.0016
Serum ammonia (per 1 $\mu\text{g}/\text{dL}$)	1.004	0.990–1.018	0.5353
BTR (per one)	0.907	0.724–1.121	0.3756
Child-Pugh A/B or C	0.726	0.416–1.261	0.2545

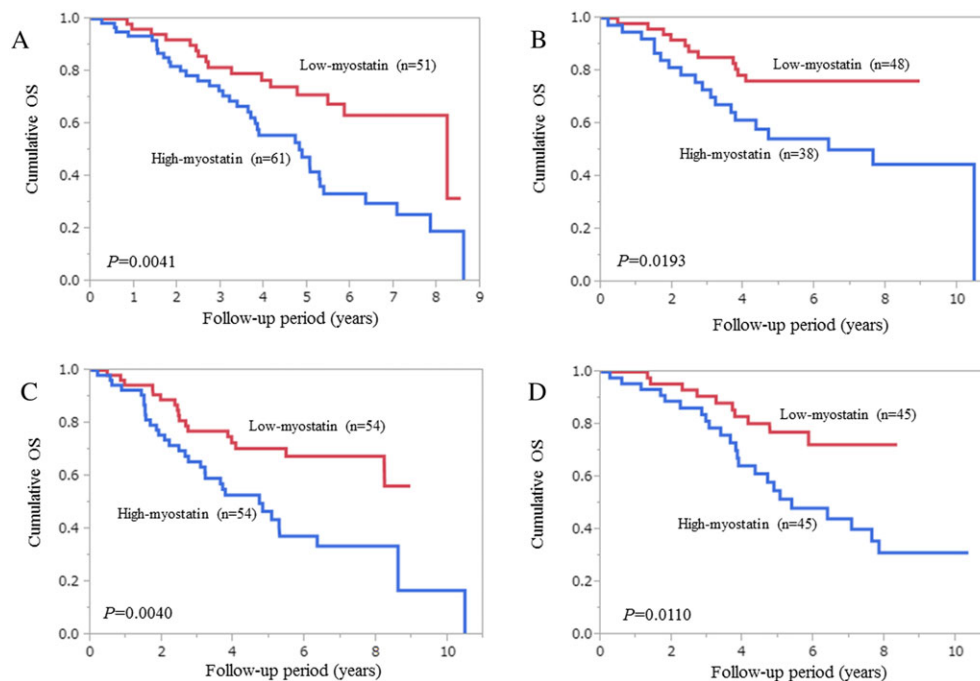
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid to tyrosine ratio; HCC, hepatocellular carcinoma; HOMA-IR, homeostasis model assessment of insulin resistance.

the high-myostatin group ($n = 33$) ($P = 0.0478$; Figure 4C). In patients with a lower PMI (less than the cut-off PMI value for each gender, $n = 99$), patients in the low-myostatin group ($n = 42$) tended to have a significantly higher survival rate than those in the high-myostatin group ($n = 66$) ($P = 0.0760$; Figure 4D).

Table 5 Comparison of baseline characteristics between the high-myostatin group and the low-myostatin group

Variables	High-myostatin group	Low-myostatin group	P value
Age (years)	68.65 (22.64–85.26)	66.54 (25.47–89.77)	0.4379
Gender, male/female	54/45	54/45	1.000
Body mass index (kg/m ²)	22.80 (13.05–36.61)	22.71 (15.34–35.88)	0.6991
PMI (cm ² /m ²), men	4.84 (2.06–8.22)	6.37 (2.28–9.68)	<0.0001
PMI (cm ² /m ²), women	3.87 (1.16–5.84)	4.25 (0.79–6.70)	0.0175
Causes of liver disease			
HBV/HCV/alloholic/others	9/56/7/27	8/60/12/19	0.4067
Child–Pugh, A/B/C	52/45/2	72/27/1	0.0206
Presence of HCC, yes/no	24/75	16/83	0.2150
Total bilirubin (mg/dL)	1.1 (0.3–4.1)	1.0 (0.3–6.0)	0.1406
Serum albumin (g/dL)	3.4 (2.1–4.5)	3.7 (2.0–4.9)	<0.0001
Prothrombin time (%)	71.4 (43.5–115.6)	78.0 (35.6–106.0)	0.0147
Platelets (×10 ⁴ /mm ³)	8.4 (2.5–39.8)	8.6 (2.6–42.4)	0.2776
Serum sodium (mmol/L)	140 (127–145)	140 (133–144)	0.5895
Serum creatinine (mg/dL)	0.69 (0.34–4.14)	0.71 (0.41–2.08)	0.6987
Total cholesterol (mg/dL)	148 (70–306)	153 (82–292)	0.7749
AST (IU/L)	48 (18–402)	38 (16–289)	0.1003
ALT (IU/L)	38 (11–487)	32 (9–233)	0.4665
Serum ammonia (μg/dL)	48 (21–132)	32 (7–77)	<0.0001
HOMA-IR	2.44 (0.07–36.41)	2.62 (0.29–21.14)	0.9941
BTR	3.49 (1.46–8.56)	4.54 (2.53–9.67)	<0.0001
Ascites, yes/no	22/77	13/86	0.1354
Alpha-fetoprotein (ng/mL)	6.3 (0.8–613.5)	5.0 (1.0–1345.0)	0.0422

Data are expressed as number or median (range). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid to tyrosine ratio; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment of insulin resistance; PMI, psoas muscle index.

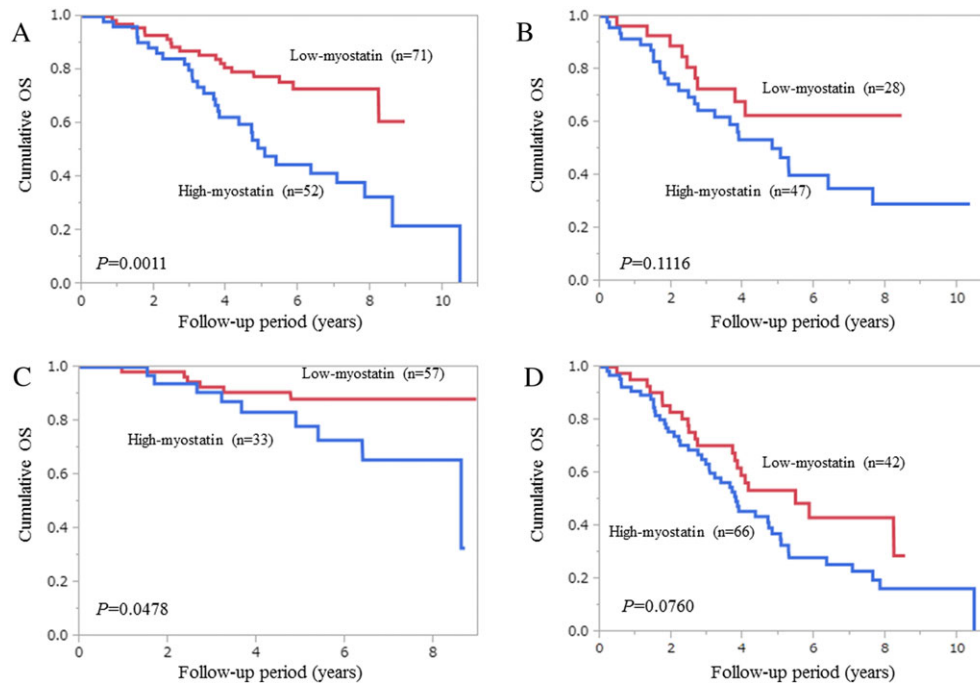
Figure 3 Subgroup analyses with regard to the influence of serum myostatin levels on survival. (A) Patients with a higher age (65 years or more, $n = 112$); (B) patients with a lower age (less than 65 years, $n = 86$); (C) male patients ($n = 108$); and (D) female patients ($n = 90$).

Interventional therapies during the follow-up period and the effects of myostatin on survival

In hepatitis B virus-related LC patients ($n = 17$), 12 patients (70.6%) received nucleoside analogue therapies during their

follow-up period. In HCV-related LC patients ($n = 116$), 41 patients achieved a sustained virological response (SVR) to direct acting antiviral therapies ($n = 18$) or interferon-based therapies ($n = 23$). In SVR patients, the difference between the high-myostatin group ($n = 14$) and the low-myostatin

Figure 4 Subgroup analyses with regard to the influence of serum myostatin levels on survival. (A) Child–Pugh A patients ($n = 123$); (B) Child–Pugh B or C patients ($n = 75$); (C) patients with a higher psoas muscle index (PMI) (greater than the cut-off PMI value for each gender (men, $6.36 \text{ cm}^2/\text{m}^2$; women, $3.92 \text{ cm}^2/\text{m}^2$), $n = 90$); and (D) patients with a lower PMI (less than the cut-off PMI value for each gender, $n = 108$).



group ($n = 27$) did not reach significance in terms of OS ($P = 0.5505$). Similarly, in non-SVR patients, the difference between the high-myostatin group ($n = 50$) and the low-myostatin group ($n = 25$) did not reach significance in terms of OS ($P = 0.5155$). However, in the remaining patients (alcoholic or others, $n = 65$), the difference between the high-myostatin group ($n = 27$) and the low-myostatin group ($n = 38$) was significant in terms of OS ($P = 0.0022$).

Discussion

To the best of our knowledge, this is the first study of its kind to examine the influence of serum myostatin level on survival in patients with LC. The expression of myostatin in skeletal muscle is increased in LC patients and believed to be associated with anabolic resistance.^{21,28,41,42} In our results, for the entire cohort, and subjects without HCC at baseline, higher baseline myostatin levels were associated with significantly worse survival, and this had marginal significance ($P = 0.0855$ for the entire cohort and $P = 0.0778$ for patients without HCC) in our multivariate analysis. In addition, similar trends were observed in the subgroups other than Child–Pugh B or C.

The reason why the difference in the two groups did not reach significance for subgroups involving Child–Pugh B or C patients is probably due to the small sample size. These results indicate that serum myostatin levels can represent an adverse predictor for LC patients. Recent molecular investigations, using muscle biopsy specimens for patients with LC and healthy controls, have demonstrated increased skeletal muscle expression and almost four-fold higher plasma concentrations of myostatin in LC patients as compared with controls.^{23,30,43} However, in our data, serum myostatin levels in patients with Child–Pugh B or C were significantly higher than those in patients with Child–Pugh A. In view of these results, serum myostatin levels appeared to be closely linked to liver functional reserve.

Current Japanese guidelines for sarcopenia in liver diseases define male patients with a $\text{PMI} \leq 6.36 \text{ cm}^2/\text{m}^2$ and female patients with a $\text{PMI} \leq 3.92 \text{ cm}^2/\text{m}^2$ as those with reduced muscle mass.⁵ When these criteria are adapted to our current data, 67 (62.0%) out of 108 male patients had reduced muscle mass, while 41 (45.6%) out of 90 female patients had reduced muscle mass. These differences in the prevalence of reduced muscle mass may be attributed to the difference of baseline serum myostatin levels between male and female patients. On the other hand, cross-sectional areas (cm^2) of skeletal

muscles at the L3 level on CT images divided by height squared (skeletal muscle index (cm^2/m^2)) are reported to be useful for predicting outcomes.⁴⁴ By using dedicated software (Tomovision, Canada), fatty transformation in the muscle can be excluded, and skeletal muscle mass at the L3 level on CT can be measured precisely, although this practice has limitations in terms of cost and accessibility. While measuring psoas muscle mass at the L3 level on CT by manual tracing is not time-consuming in clinical settings and is readily available, it may have the disadvantage of lacking accuracy as compared with muscle mass measurement by dedicated software.³¹

Hyperammonemia has recently attracted much attention as a possible mediator in the liver–muscle axis, and the related up-regulation of myostatin is regarded as a mechanism of impaired protein synthesis and increased autophagy, which is linked to the development of sarcopenia.^{10,24–26,42,45} In our data, serum myostatin significantly correlated with serum ammonia levels and inversely correlated with PMI irrespective of gender. These observations were in line with previous reports and strongly support the hypothesis that skeletal muscle mass depletion can occur via impaired ammonia detoxification and its related higher myostatin expression.^{10,24–26,42,45} The significant inverse correlation between serum myostatin level and serum albumin level and BTR in our results may be linked to these earlier observations. In particular, the inverse relationship between myostatin level and BTR suggested that muscle mass loss in LC patients developed via a reduction in protein synthesis.^{3,12–14} On the other hand, because skeletal muscle is predominantly responsible for postprandial glucose disposal, insulin resistance in the skeletal muscle can result in substantial metabolic disturbances.²⁸ In addition, as described earlier, myostatin deficiency has more recently been demonstrated to exert beneficial effects on insulin sensitivity.^{28,29} However, in this study, serum myostatin levels did not significantly correlate with homeostasis model assessment of insulin resistance in male or female patients. The reasons for this are unclear, and further investigations will be needed to confirm these results.

In HCV-related LC subjects, serum myostatin levels did not exert influence on survival irrespective of achieving SVR. However, in non-B and non-C (NBNC) LC subjects, the influence of serum myostatin levels on survival was prominent. These differences may be attributed to differences in the cause of death for HCV-related LC subjects and NBNC LC subjects. Out of 23 NBNC LC mortalities, 18 patients (78.3%) died from liver failure, while out of 52 HCV-related LC mortalities, 23 patients (44.2%) died from liver failure. As described previously, serum myostatin levels are associated with liver functional reserve; thus, patients

with higher myostatin levels tended to die from liver failure rather than tumour progression.

Skeletal muscle fatigue can contribute to impaired exercise capacity, and there is compelling evidence that muscle mass loss is associated with worse survival; there are also limited clinical data demonstrating that the improvement of muscle mass in LC patients can lead to favourable clinical outcomes.^{8,10,22} Thus, reversing skeletal muscle mass is a priority field in therapeutic strategies for LC subjects. Therapeutic advances will thus be able to correct adverse consequences in LC patients. Considering our current results, myostatin antagonists such as follistatin may have the potential to benefit muscle protein turnover; however, the influence of such agents on clinical outcomes have not yet been fully examined, and additional investigations will be required.^{22,29,30}

Several limitations need to be acknowledged in our current analysis. Firstly, this study was a single centre retrospective observational study utilizing data acquired from stored serum samples. Secondly, parameters reflecting muscle dysfunction, such as a reduction in hand-grip strength, were not included into our analysis. In future studies, the relationship between skeletal muscle function and serum myostatin level should be examined further. Thirdly, the number of Child–Pugh C patients was very small, and our results cannot be applied to such patients. Finally, during the observation period, various interventional therapies for underlying liver diseases were performed in each patient, potentially leading to bias. However, our study results suggested that higher serum myostatin levels correlated with muscle mass loss, hyperammonemia, and impaired protein synthesis as reflected by lower serum albumin level and lower BTR, and higher serum myostatin levels were associated with adverse clinical outcomes for LC patients.

In conclusion, serum myostatin represents a promising target for predicting the clinical outcomes of patients with LC.

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Conflicts of interest

None of the authors has any conflicts of interest to declare.

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