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Original articles

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PROGNOSTIC USEFULNESS OF SERUM MYOSTATIN IN ADVANCED CHRONIC LIVER DISEASE: ITS RELATION TO GENDER AND CORRELATION WITH INFLAMMATORY STATUS

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In hospitalized patients with advanced chronic liver disease (ACLD), we aimed to evaluate the association between myostatin and muscle mass, its relation to inflammation and to assess the added prognostic value of myostatin for survival. In a prospective single-center cohort study, inclusion criteria were: consecutive hospitalization for ACLD and Child-Pugh score ≥ 7 points. Baseline parameters were myostatin, C-reactive protein (CRP), hand-grip strength (HGS), mid-arm muscle circumference (MAC), transversal psoas muscle index (TPMI). Patients were followed-up for at least 400 days. We included 198 men, 157 women, and 40 controls, median myostatin levels in pg/ml were 1790.1 in women, 1959.4 in men, and 3850.0 and 2996.o in healthy men and women. Myostatin positively correlated with TPMI, but weakly with MAC and HGS, and not in women. Myostatin negatively correlated with CRP in both genders. In cases with CRP 10 mg/l, regression analysis of myostatin versus HGS, MAC or TPMI showed steeper dependence curve. During follow up, 85 men and 64 women (42.9% versus 40.8%) have died, 22 men and 19 women (11.1% versus 12.1%) underwent liver transplantation. Cumulative incidence of death was higher in men with myostatin levels < 1600.0 pg/ml, but not in women. In men, MELD score and myostatin cut-off were independent predictors of worse survival but did not predict survival in women. In men, myostatin levels directly reflect the muscle mass and low levels independently predict worse survival. In women, myostatin is not associated with muscle mass or survival.

Key words: *advanced chronic liver disease, myostatin, C-reactive protein, hand-grip strength, inflammation, mid-arm muscle circumference, transversal psoas muscle index*

INTRODUCTION

Myostatin is a member of the transforming growth factor beta (TGF-β) family. It is expressed in the skeletal muscle and serves as a natural inhibitor of muscle growth. Several reports have confirmed that myostatin synthesis is upregulated in advanced chronic liver disease (ACLD), presumably due to increased levels of ammonia (1). Studies on the downstream mechanisms of intracellular myostatin action have shown that myostatin promotes autophagy and muscle wasting (2). Sarcopenia has been proven to adversely affect the outcome of ACLD (3, 4). However, the diagnosis of sarcopenia in ACLD is challenging. Recent reports have proposed the use of imaging as the gold standard for diagnosing sarcopenia in liver transplant candidates (5, 6). This approach requires CT imaging and subsequent computer-assisted image analysis. These techniques are rather time-consuming and not widely available. Therefore, in the management of ACLD there is an unmet need for a simple serum marker reflecting total muscle mass and having a clinically relevant prognostic value. One recent study in patients with stable ACLD has shown, that increased serum myostatin was associated with lower muscle mass and adverse prognosis (7). In decompensated ACLD (dACLD), the diagnosis of sarcopenia is even more complex, yet it has been shown to be an independent predictor of the adverse outcome on the liver transplantation waiting list (8), during hospitalization (9) and following liver transplantation (10). Since myostatin is a member of TGF-β family, its levels in dACLD may be affected by organ dysfunction or systemic inflammation (11). The usefulness of myostatin in decompensated ACLD with possible relation to systemic inflammation has not been studied yet. We therefore hypothesize that serum myostatin might be useful in identifying dACLD patients with low muscle mass and overall worse prognosis. Our first aim was to evaluate the association of baseline myostatin with muscle mass parameters and other common laboratory markers as well as to compare myostatin values in patients with ACLD with healthy controls. Our second goal was to explore the possible association between myostatin and inflammatory status. Third, our aim was to assess the added prognostic value of myostatin for overall survival of patients diagnosed with dACLD.

PATIENTS AND METHODS

Patients

We designed a prospective observational single-center cohort study which included consecutive patients with ACLD who were hospitalized during an inclusion period of 33 months (from June 2014 to February 2017) at the Hepatology, Gastroenterology and Liver Transplantation (HEGITO) Unit, FD Roosevelt Faculty Hospital in Banska Bystrica, Slovakia.

The following inclusion criteria were defined: 1) hospitalization for decompensated ACLD or for curative hepatocellular carcinoma (HCC) management, or for evaluation for liver transplantation due to ACLD; 2) all included patients had to have a Child-Pugh class B or C (≥ 7 points). Patients hospitalized for elective procedures, those with Child-Pugh class A, patients with HCC outside of the Milan criteria or with known malignancy at other organs or terminal disease were excluded (12). Healthy controls were recruited among volunteers who underwent anthropometric measurements and blood sampling for myostatin concentration.

The study has been carried out in accordance with the provisions of the Declaration of Helsinki and has been approved by the local ethical committee (Eticka komisia FNsP F.D. Roosevelta, Nam. L. Svobodu 1, 975 17 Banska Bystrica) on May 21, 2014. All study participants signed informed consent for participation in the study as well as for the presentation of the study results. Due to the non-interventional nature of the study, it has not been registered at the clinicatrials.gov.

Experimental procedures

At baseline, all patients were evaluated for their presumed etiology of ACLD, cirrhosis complications, common laboratory parameters: full blood count, renal function tests, serum ammonia, C reactive protein (CRP) and liver synthetic function; daily urinary creatinine excretion and the Child-Pugh and Model for end-stage liver disease (MELD) score. Body mass index, mid-arm circumference (MAC, in cm), tricipital skinfold (TSF, in mm) and hand-grip strength (HGS, in kg) were measured by a trained nurse (JV). Dynamometer KERN MAP-80K1 was used to measure the HGS. Parameters defining

Table 1. Summary statistics and gender specific baseline characteristics of the control group (n = 40) and the study population (n = 355).

Numerical parameters (normal range)	**Control group Men** n = 5 median [IQR*]	**Control group Women** n = 35 median [IQR*]	**Men** n = 198 median [IQR*]	**Women** n = 157 median [IQR*]	**P** value	**Z** value
Age (years)	30.0 [26.3, 33.0]	42.0 [32.5, 47.0] II	56.3 [48.5, 61.7]	56.0 [45.2, 61.5]	0.518	0.647
Body mass index (18.5 - 24.9 kg/m^2)	26.9 [25.2, 28.9]	24.8 [22.7, 27.4]	27.1 [24.7, 30.1]	24.7 [21.6, 29.2]	0.001	3.466
Child-Pugh score			10.0 [8.0, 11.0]	10.0 [8.0, 11.0]	0.748	0.321
Model for end-stage liver disease score			17.0 [13.0, 21.8]	16.0 [13.0, 20.0]	0.328	0.979
C-reactive protein at baseline (< 5 mg/l)			12.5 [6.8, 31.0]	13.8 [5.3, 27.4]	0.608	0.512
White blood cells (3.8 – 10 × 109/L)			5.9 [4.2, 9.4]	5.5 [3.6, 9.3]	0.186	1.322
Lymphocytes (0.8 – 4.08 × 109/L)			1.2 [0.7, 1.7]	1.1 [0.7, 1.8]	0.676	0.418
Serum creatinine (M: 62-106, F: 44-80 μmol/L)			79.5 [61.0, 105.8]	61.0 [48.0, 82.0]	<0.001	5.279
Urea (M: 2.8-8.0, F: 2.0-6.7 mmol/L)			6.1 [4.1, 10.1]	4.9 [3.7, 7.4]	0.002	3.121
Urinary creatinine (M: 9-21, F: 7-14 mmol/24 h)			9.8 [6.2, 15.3]	7.00 [4.8, 12.4]	0.007	2.704
Hand-grip strength (M: >30 kg, F: >20 kg)	55.2 [51.7, 58.6]	26.8 [25.1, 31.8] II	28.5 [23.7, 36.2]	17.6 [13.7, 21.7]	<0.001	11.29
Mid-arm circumference (cm)	32.0 [30.3, 34.3]	28.0 [26.0, 31.0] II	27.0 [25.0, 30.0]	25.0 [22.0, 28.0]	<0.001	5.119
Tricipital skinfold (mm)	11.2 [6.2, 15.2]	18.0 [14.2, 23.5] II	11.0 [7.2, 17.5]	13.6 [8.6, 20.0]	0.020	2.324
Transversal psoas muscle index (cm/m) ‡			17.8 [15.2, 20.7]	15.7 [13.3, 17.7]	<0.001	3.952
Myostatin baseline (pg/ml) §	3850 [3289, 5909]	2996 [2482, 4581]	1959.4 [1082.8, 3914.8]	1790.1 [914.1, 3158.7]	0.143	1.466
Median follow up (days)			248.7 [65.0, 360.0]	256.6 [69.0, 375.0]	0.743	0.216

Consecutive cases hospitalized for decompensated advanced chronic liver disease, n = 355.
* interquartile range, † difference between study group men and women, ‡ n = 186, 110 men, 76 women; § difference between control group and the study population, P < 0.001 for both genders; II difference between genders in the control group, P < 0.05. M, males; F, women.

central muscle mass were read from computed tomography (CT) scans in patients who underwent imaging during their hospital stay. Axial and transversal dimensions of the right major psoas muscle at the level of L3 were measured offline by one trained radiologist (MZ) blinded to other baseline parameters. Measured parameters were used to calculate a transversal psoas muscle index (TPMI = right transversal psoas diameter (mm)/body height (m)) as defined by Durand *et al*., and gender-specific cut-offs by Praktiknjo *et al*., (13, 14). We used two definitions of sarcopenia as described previously: A) low HGS (men < 30 kg, women < 20 kg) (15) as a marker of low muscle strength; B) transversal psoas muscle index (TPMI) lower than the predefined cut-off < 16.8 mm/m (13) and gender-specific cut-offs 17.8 mm/m for men and 14.0 mm/m for women (14) as a marker of central muscle loss. TPMI could only be measured in a subgroup of 186 cases (110 men, and 76 women) with available CT scans. To define an inflammatory status, we used a CRP cut-off of 10 mg/dl, which has been previously reported as a sensitive marker for cirrhosis associated bacterial infections (16) and mortality (17).

Serum myostatin was analyzed from fasting morning blood samples on the second or third day of hospital stay using a commercially available kit GDF-8 myostatin immunoassay, R&D systems, Minneapolis, USA. Normal serum values in humans were defined as 4206.0 ± 1906.0 pg/ml.

After the enrollment at initial hospitalization, we followed patients up after one month, and at months 3, 6, 9, and 12. For the data analysis, we considered the follow up terminated when death or liver transplantation occurred at least 400 days after inclusion. Patients with any of the events were censored on the day of the event. Survival status has been verified using the national registry of deceased inhabitants. Our aim was to get access to all death certificates, but we were able to ascertain the cause of death only in 70 out of 149 deceased patients.

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018, licensed to Tomas Koller) and R v.3.5.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/, GNU general public license). All parameters were evaluated for normal distribution by Kolmogorov-Smirnov test, results of non-parametric data are presented as proportions or median and interquartile range. Numerical parameters were compared using the Mann-Whitney test or (two groups) or Kruskal Wallis test (more groups) and categorical variables by chi-square test. We used Spearman rank correlation and linear regression to assess the relationship between myostatin and other numerical variables. Predictive value of serum myostatin in the prediction of survival and myostatin cut-off values were calculated using an area under receiver operating curve (AUROC, DeLong *et al*. (18)) as well as by using a locally estimated scatterplot smoothing (LOESS) curve for both sexes separately. Predictive value of myostatin below or above the cut-off in the prediction of overall survival was evaluated using Fine-Gray proportional hazard regression and cumulative incidence function for competing events. Death was selected as an event of interest and liver transplantation as the competing event. This test was carried out in univariate and multivariate analysis using an EZR package for R (19). The same package has been used to calculate the sufficient sample size. At predicted hazard ratio of 1.5 with expected at least 20% difference in overall survival curves, the statistical power of 0.8 and P < 0.05, the minimal sample size was set for 110 cases for each gender. A significant association between the study variables was considered at the level of null-hypothesis probability inferior to 0.05.

RESULTS

Summary statistics

During the inclusion period 3041 patients were hospitalized at the HEGITO unit, 424 were identified as patients of interest having decompensated ACLD. 69 patients were excluded for not fulfilling the inclusion criteria (Child-Pugh A 29 patients, advanced HCC 31 patients, elective hospitalization 9 patients). Our final study cohort consisted of 198 men and 157 women (n = 355) and 40 healthy controls. Comparison of baseline characteristics between genders is displayed in *Table 1* and *Table 2*. Female patients had significantly lower BMI and lower parameters of mean muscle mass and hand-grip strength. Alcoholic etiology of ACLD was more common in men, whereas fatty liver and cholestatic or autoimmune disease were more prevalent in women. Age, Child-Pugh and MELD scores or survival were not different between the genders. In the final cohort of patients, none was diagnosed with HCC.

Myostatin baseline and correlations

Median baseline myostatin concentration in pg/ml in men and women was 1959.4 and 1790.1 (P = 0.143) respectively versus 3850.0 in healthy men and 2996.0 in healthy women (P < 0.001). Myostatin concentration in patients with alcoholic liver disease had the lowest median compared with cholestatic liver disease, non-alcoholic fatty liver and viral hepatitis (1691.8 versus 1849.0, 2644.0 and 2373.0), but the difference did not reach the statistical significance (Kruskal Wallis test, P = 0.081). In men, we found positive correlations between serum myostatin and TPMI and weaker correlations with MAC and HGS. In women, myostatin weakly correlated with TSF and HGS, but not with MAC or TPMI. In both genders, we found a significant negative correlation between myostatin and CRP, *Fig. 1*) and white blood cell count. For more details, see *Table 3*.

Myostatin, muscle parameters, and inflammation

Since we observed a significant correlation between myostatin and muscle parameters and myostatin and markers of inflammation, we performed a regression analysis stratified for the baseline CRP. Linear regression between myostatin and HGS in patients with CRP ≤ 10 mg/l (*Fig. 2*.) showed a significantly steeper dependence curve compared with patients with CRP > 10 mg/l. A similar pattern has been observed between myostatin and mid-arm circumference (*Fig. 3*) and myostatin and TPMI. Patients with CRP ≤ 10 mg/l had significantly steeper curves compared with cases with CRP > 10 mg/l (MAC, slope 222.6 ± 40.4, P < 0.001 versus slope 80.9 ± 26.3, P = 0.003, difference 141.0 ± 46.8, P = 0.003 and TPMI, slope 289.7 ± 64.3, P < 0.001 versus slope 75.7 ± 60.1, P = 0.212, difference 214.0 ± 89.8, P = 0.018).

Myostatin in the prediction of survival

During follow up, death occurred in 85 male patients (42.9%) and 64 female patients (40.8%). Liver transplantation was carried out in 22 men (11.1%) and 19 women (12.1%). Survival probability in men and women was 87.0% and 85.1% at 30 days, and 75.7% and 75.3% at 90 days respectively.

Using the receiver operating curve (ROC) analysis, we found that myostatin in men could moderately predict survival at 30 and 90 days (*Fig. 4A* and *4B*). In women, myostatin did not predict survival at 30 and 90 days (*Fig. 4C* and *4D*). According to the ROC function, we identified the best myostatin cut-off for survival prediction for men at 1600.0 pg/ml and for women at 2300.0 pg/ml. To illustrate the association between myostatin

Table 2. Summary statistics and baseline characteristics of the study population.

Categorical parameters		**Men** n = 198* (%)	**Women** n = 157* (%)	**P-value Chi^2**
Child-Pugh stage	B C	82 (41.6) 115 (58.4)	73 (46.5) 84 (53.5)	0.389 0.840
Etiology alcohol	yes no	169 (85.4) 29 (14.6)	88 (56.1) 69 (43.9)	<0.001 37.520
Etiology cholestatic disease	yes no	9 (4.5) 189 (95.5)	22 (14.0) 135 (86.0)	0.002 9.820
Etiology of nonalcoholic fatty liver disease	yes no	17 (8.6) 181 (91.4)	32 (20.04) 125 (79.6)	0.002 10.210
Etiology viral	yes no	14 (7.1) 184 (92.9)	10 (6.4) 147 (93.6)	0.835 0.070
Hepatic encephalopathy West Haven stage	0 1 2 3 4	114 (64.4) 49 (27.7) 12 (6.8) 2 (1.1) 0 (0.0)	84 (65.6) 38 (29.7) 5 (3.9) 0 (0.0) 1 (0.8)	0.476 4.050
Sarcopenia (low hand grip strength)	yes no	108 (54.5) 90 (45.5)	105 (66.9) 52 (33.1)	0.022 5.530
Sarcopenia (low transversal psoas muscle index) Men: n=110, Women: n=76 Cut-off Durand *et al*. Cut-off Praktiknjo *et al*.		 41 (37.3) 55 (50.0)	 52 (68.4) 22 (28.9)	 <0.001 0.004
Myostatin < cut-off (pg/ml) Men < 1600, Women < 2300	yes no	67 (38.5) 107 (61.5)	83 (59.7) 56 (40.3)	<0.001 13.890
C-reactive protein > 10 mg/l	yes no	116 (58.6) 82 (41.4)	90 (57.3) 67 (42.7)	0.829 0060
Cirrhosis complications at alcohol baseline	hepatitis ascites encephalopathy infection alone not reported none varices	31 (15.7) 45 (22.7) 14 (7.1) 7 (3.5) 20 (10.1) 38 (19.2) 43 (21.7)	17 (10.8) 31 (19.7) 5 (3.2) 6 (3.8) 17 (10.8) 52 (33.1) 29 (18.5)	
Acute-on-chronic liver failure grade	0 1 2 3	136 (84.5) 13 (8.1) 6 (3.7) 6 (3.7)	106 (85.5) 8 (6.5) 6 (4.8) 4 (3.2)	0.917 0.520
Acute kidney injury grade	0 1 2 3	125 (80.1) 14 (9.0) 6 (3.8) 11 (7.1)	101 (88.6) 5 (4.4) 5 (4.4) 3 (2.6)	0.175 5.060
Endpoint	censored liver transplant death	91 (46.0) 22 (11.1) 85 (42.9)	74 (47.1) 19 (12.1) 64 (40.8)	0.909 0.200
30-day mortality	yes no	25 (13.0) 167 (87.0)	23 (14.9) 131 (85.1)	0.641 0.260
90-day mortality	yes no	45 (24.3) 140 (75.7)	37 (24.7) 113 (75.3)	1.000 0.010

*Consecutive cases hospitalized for decompensated advanced chronic liver disease, n = 355.

and mortality graphically, we also provide a locally estimated scatterplot smoothing (LOESS) curve for both sexes displayed in *Fig. 5*. In men, the curve initially runs in a descending direction compared to women, where it runs parallel to the horizontal axis.

The cumulative incidence of death or liver transplantation during follow-up stratified by the myostatin cut-off for men and women is displayed in *Fig. 6* and *7*. Figures show that in men with myostatin below the cut-off (low myostatin), the cumulative incidence of death was significantly higher compared to those with myostatin above the cut-off ($P < 0.001$). We also observed a trend towards the lower cumulative incidence of liver transplantation ($P = 0.076$) in cases with low myostatin. In women with low myostatin, we observed no

Table 3. Correlation of serum myostatin (pg/ml) with commonly observed laboratory parameters. Cases hospitalized for decompensated liver disease, n = 355.

Parameters	*	**Men**	**Women**
Age (years)	rho P value	–0.020 0.791	0.113 0.188
Body mass index (kg/m^2)	rho P value	0.194 0.010	0.032 0.718
Child-Pugh score	rho P value	–0.163 0.033	–0.068 0.428
Model for end-stage liver disease score	rho P value	–0.118 0.121	–0.203 0.018
C-reactive protein at baseline (mg/l)	rho P value	–0.438 <0.001	–0.318 <0.001
White blood cells ($\times 10^9$/L)	rho P value	–0.392 <0.001	–0.420 <0.001
Lymphocytes ($\times 10^9$/L)	rho P value	0.136 0.084	–0.198 0.027
Serum creatinine (mmol/L)	rho P value	–0.112 0.142	0.028 0.745
Serum urea (mmol/L)	rho P value	–0.194 0.010	–0.220 0.010
Urine creatinine (mmol/24 h)	rho P value	0.185 0.044	–0.103 0.316
Hand grip strength (HGS, kg)	rho P value	0.284 <0.001	0.279 0.001
Mid-arm circumference (MAC, cm)	rho P value	0.355 <0.001	0.156 0.070
Tricipal skin fold (TSF, mm)	rho P value	0.262 <0.001	0.211 0.014
Transversal psoas muscle index (TPMI, cm/m), Men: n=110, Women=76	rho P value	0.464 <0.001	0.050 0.700

*Spearman rank correlation coefficient and P-value.

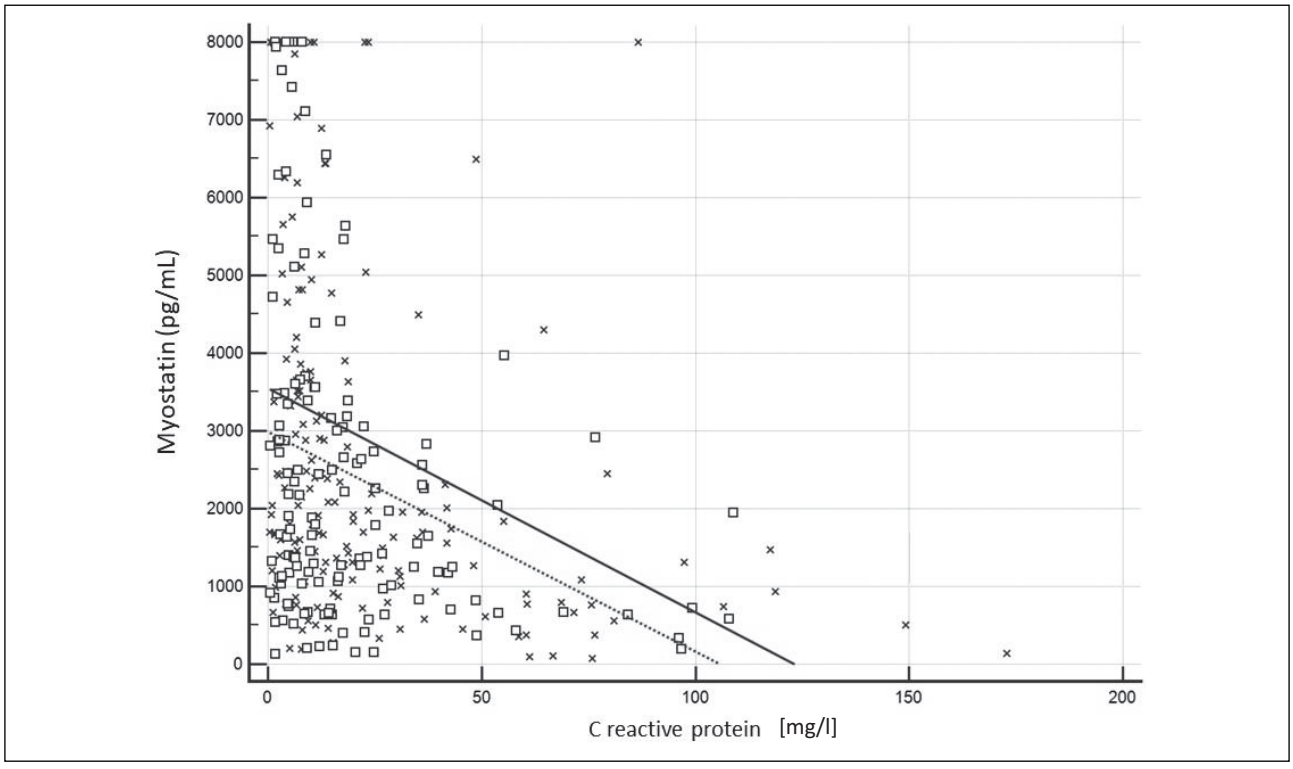

Fig. 1. Linear regression between serum myostatin and C-reactive protein at baseline in 355 patients with decompensated advanced chronic disease. Crosses and full line denote men (rho = –0.438, $R^2 = 0.1025$, $P < 0.001$, squares and dotted line denote women (rho = –0.318, $R^2 = 0.114$, $P < 0.001$).

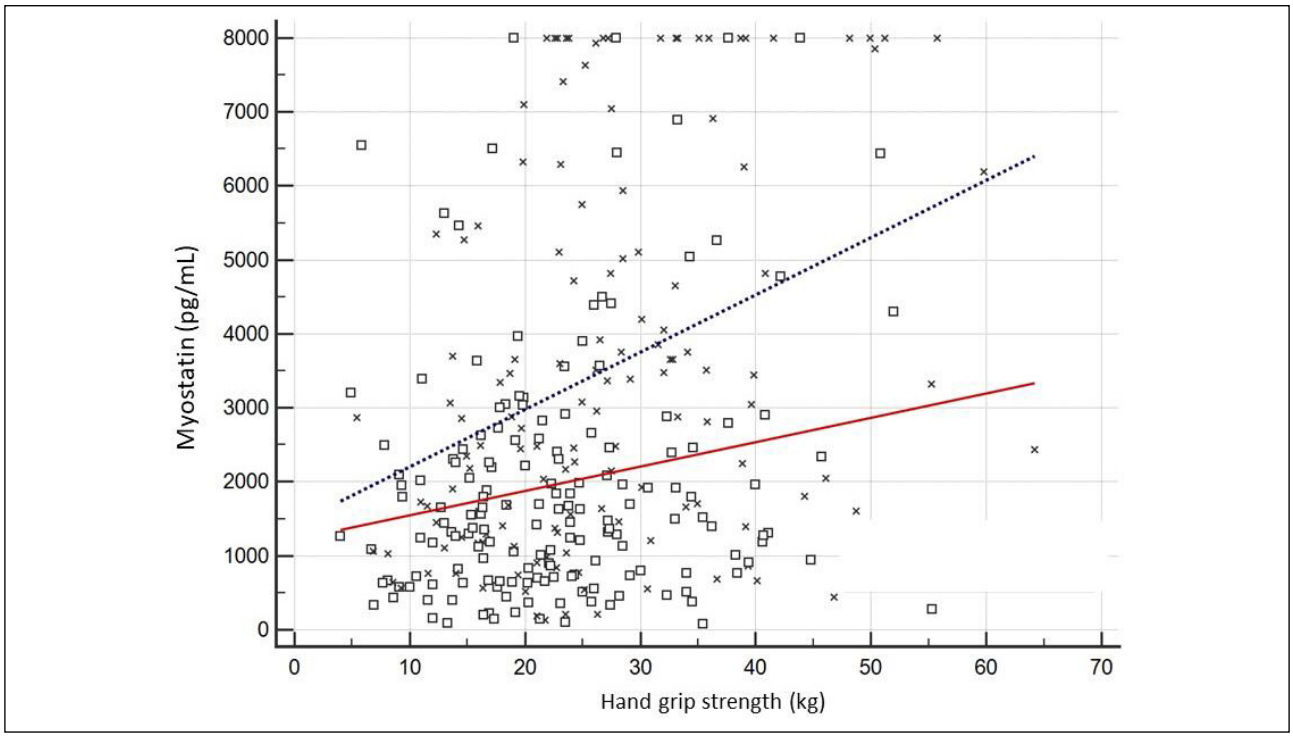

Fig. 2. Linear regression between serum myostatin and hand-grip strength stratified by the baseline C-reactive protein (CRP). Crosses and dotted line denote CRP ≤ 10 mg/l, slope 77.59 ± 18.28, P < 0.001; squares and full line denote CRP > 10 mg/l, slope 32.97 ± 13.25, P = 0.014; slope difference 44.64 ± 22.33, P = 0.046.

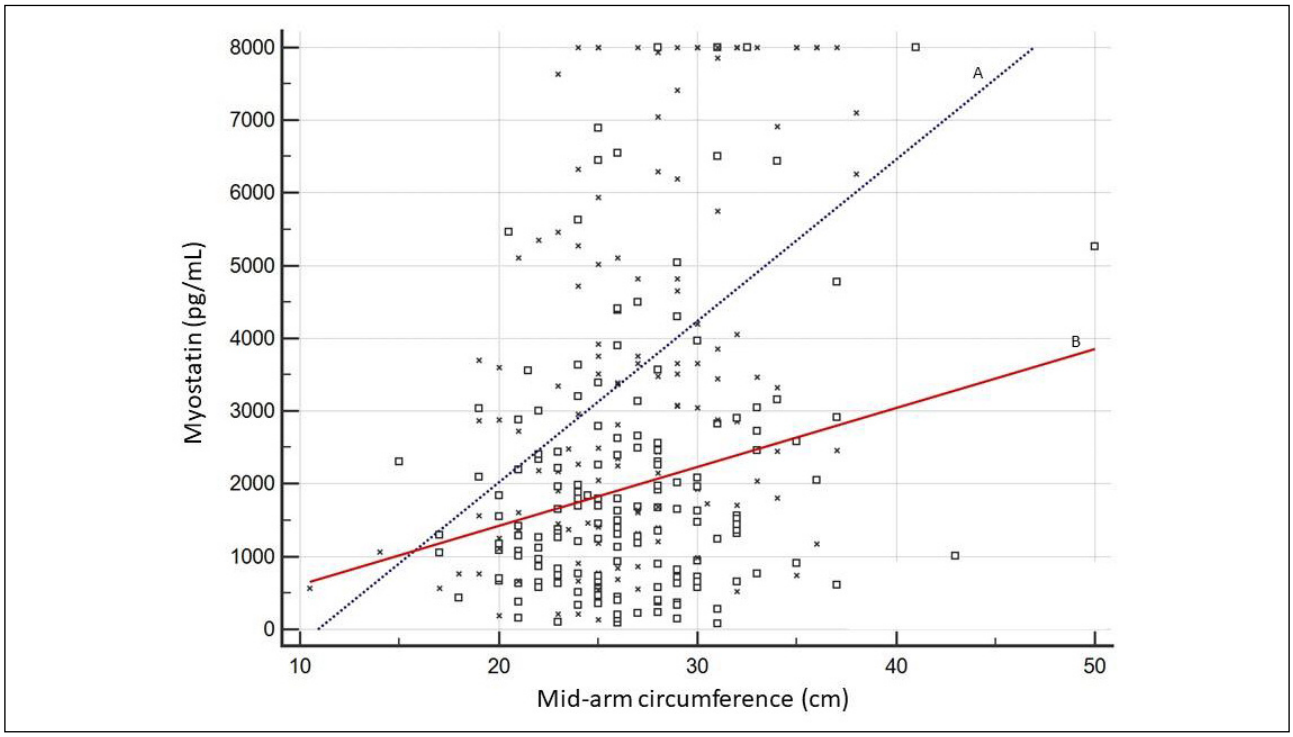

Fig. 3. Linear regression between myostatin and mid-arm circumference stratified by the baseline C-reactive protein (CRP). Crosses and dotted line denote CRP ≤ 10 mg/l, slope 222.6 ± 40.4, P < 0.001; squares and full line denote CRP > 10 mg/l, slope 80.97 ± 26.35; slope difference 141.0 ± 46.83, P = 0.003.

difference in the cumulative incidence of death (P = 0.369) or liver transplantation (P = 0.289).

To address the effect of myostatin on overall survival in comparison to other clinically available variables, we performed a univariate and multivariate Fine-Gray proportional hazard regression for competing events, see details in *Table 4*. In men, overall survival was significantly affected by Child-Pugh and MELD scores, CRP, white blood cell count, HGS, MAC, TPMI,

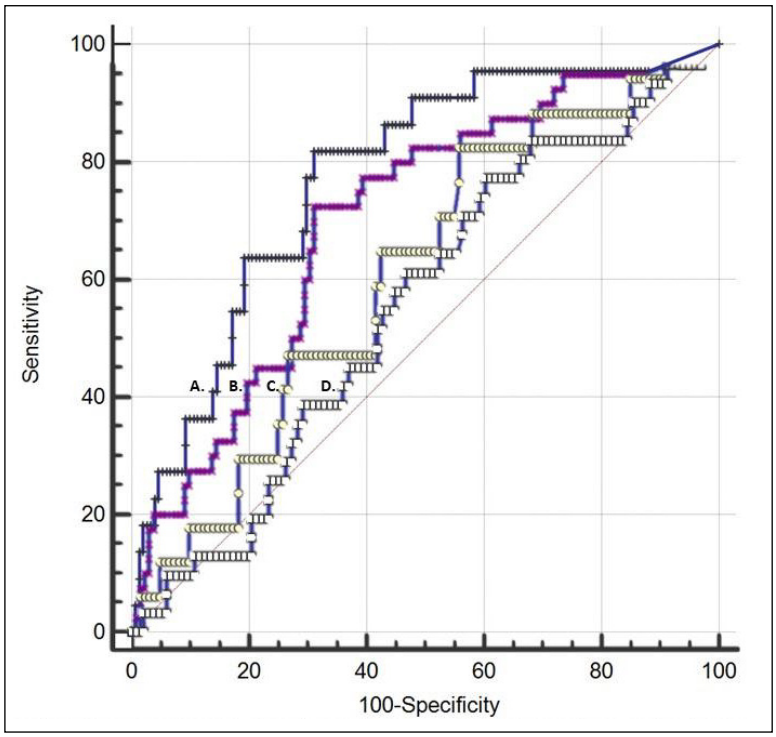

Fig. 4. Receiver operating curves (ROC) for myostatin as a predictor for survival in decompensated advanced chronic liver disease. A: 30-day survival in men AUROC = 0.774 (0.705 – 0.834), P < 0.001. B: 90-day survival in men, AUROC = 0.703 (0.629 – 0.771), P < 0.001; C: 30-day survival in women, AUROC = 0.609 (0.522 – 0.691), P = 0.12. D: 90-day survival in women AUROC = 0.555 (0.467 – 0.641), P = 0.333. Best identified cut-off for myostatin: men 1600 pg/ml, women 2300 pg/ml.

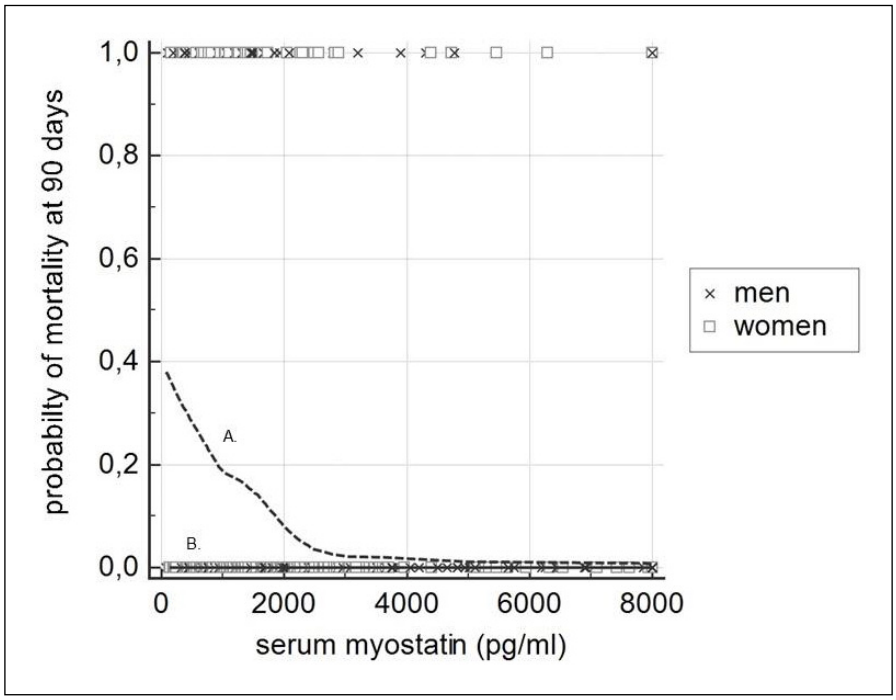

Fig. 5. Association of myostatin concentration (pg/ml) and mortality at 90 days using a locally estimated scatterplot smoothing (LOESS) curve with a smoothing span set to 50%. A: dotted line for men; B: solid line for women.

and myostatin. In women, overall survival was influenced by age, Child-Pugh and MELD scores, CRP, white blood cell count, and HGS.

In the multivariate analysis, we constructed two gender-specific models. Forest plot of the results for both models is displayed in *Fig. 8*. In men, MELD score (HR = 1.074, 95%CI 1.028 – 1.122, P = 0.001) and low myostatin (HR = 1.832, 95%CI 1.099 – 3.056, P = 0.02) were significant predictors of overall survival, while age, low HGS or CRP > 10 mg/l had no significant impact. In women, age (HR = 1.037, 95%CI 1.008 – 1.066, P = 0.013) and MELD (HR = 1.122, 95%CI 1.060 – 1.187, P < 0.001) were independent predictors of overall survival, but not low HGS, low myostatin or CRP > 10mg/l.

DISCUSSION

In the current study, we found that in ACLD serum myostatin concentrations have a gender-specific interpretation. In men, baseline myostatin positively correlates with parameters of muscle mass, however, the association is significantly weakened by the increased inflammatory response. Myostatin

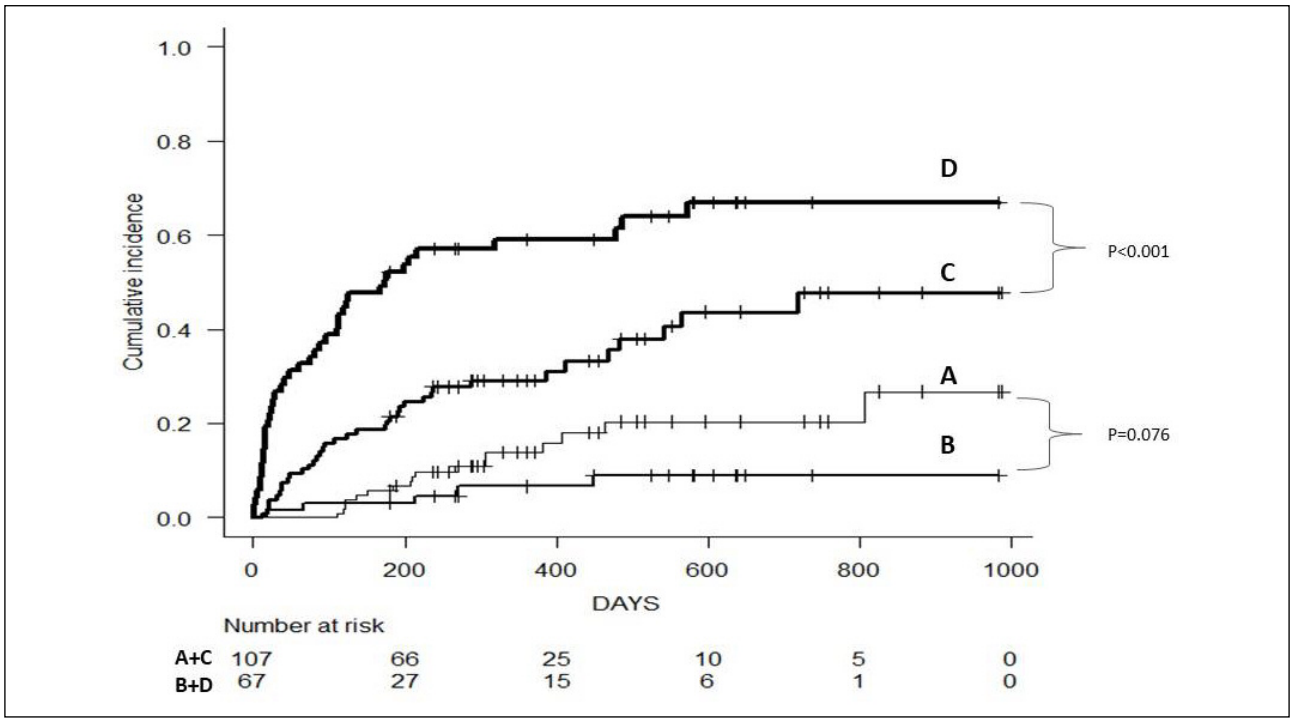

Fig. 6. Cummulative incidence of competing events (liver transplantation or death) and Gray test in men with decompensated advanced chronic liver disease stratified by myostatin levels. A: myostatin ≥ cut-off, event: liver transplantation. B: myostatin < cut-off, event: liver transplantation. Difference between A and B, P = 0.076. C: myostatin ≥ cut-off, event: death. D: myostatin < cut-off, event: death. Difference between C and D, P < 0.001 (cut-off for myostatin: men < 1600 pg/ml.

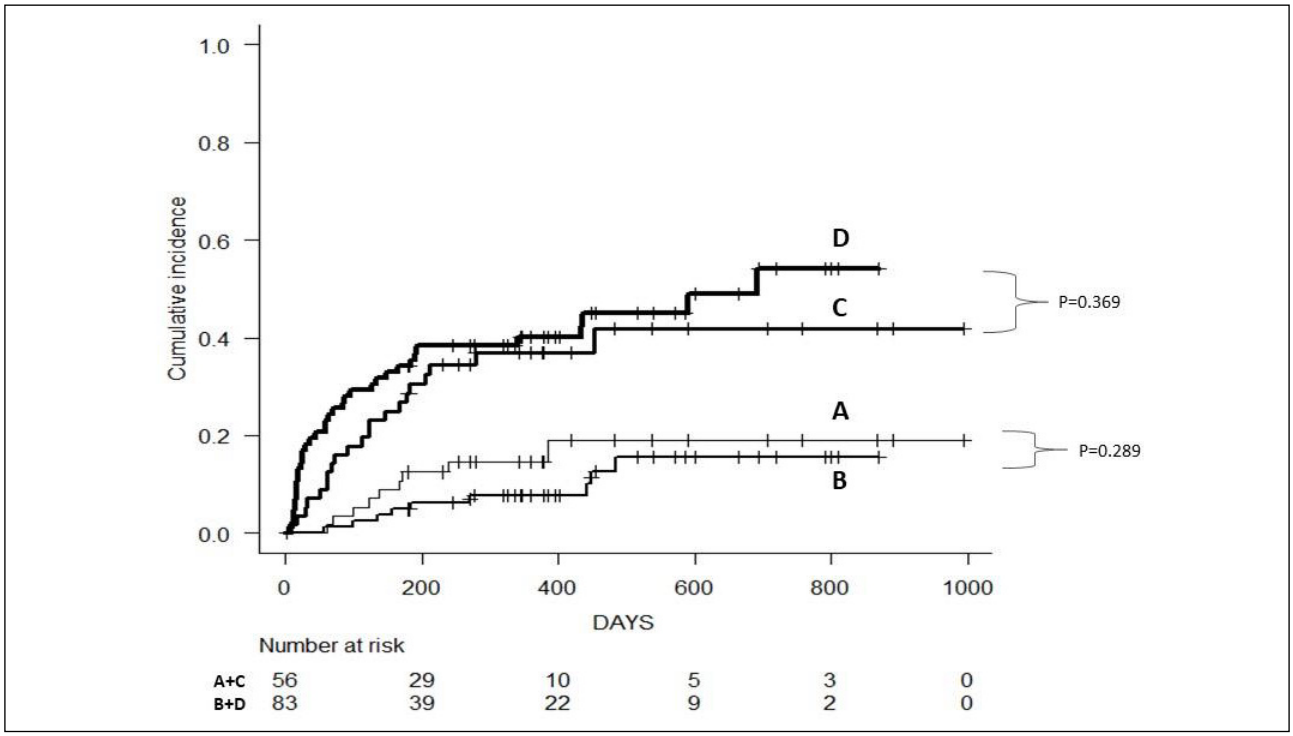

Fig. 7. Cummulative incidence of competing events (liver transplantation or death) and Gray test in women with decompensated advanced chronic liver disease stratified by myostatin levels. A: myostatin ≥ cut-off, event: liver transplantation. B: myostatin < cut-off, event: liver transplantation. Difference between A and B, P = 0.289. C: myostatin ≥ cut-off, event: death. D: myostatin < cut-off, event: death. Difference between C and D, P = 0.369 (cut-off for myostatin: men < 2300 pg/ml.

below the identified cut-off is independently associated with worse overall survival. In women, myostatin levels do not predict survival.

In patients with ACLD muscle wasting is an independent predictor of adverse outcome. Gold standard tests for evaluating muscle mass require a CT scan and image processing, which

Table 4. Hazard ratios of gender specific predictors of overall survival in 355 acute on chronic liver fdisease patients using Fine-Gray proportional hazard regression for competing events. Univariate analysis: event of interest: death, competing event: liver transplantation.

Parameters	Men		Women	
	HR	**95%CI**	**HR**	**95%Cl**
Age (years)	1.013	0.994 – 1.033	1.030	1.005 – 1.056
Body mass index (kg/m^2)	0.994	0.955 – 1.034	1.019	0.971 – 1.069
Child-Pugh score	1.313	1.160 – 1.480	1.545	1.305 – 1.829
Model for end-stage liver disease score	1.092	1.049 – 1.137	1.132	1.083 – 1.184
C-reactive protein baseline (mg/l)	1.013	1.007 – 1.020	1.012	1.002 – 1.021
White blood cells (×10^9/L)	1.055	1.004 – 1.109	1.052	1.020 – 1.084
Lymphocytes (×10^9/L)	0.797	0.512 – 1.239	0.882	0.707 – 1.100
Urinary creatinine (mmol/24 h)	0.984	0.951 – 1.018	0.993	0.954 – 1.033
Hand grip strength (kg)	0.955	0.932 – 0.980	0.936	0.897 – 0.978
Mid-arm circumference (cm)	0.951	0.904 – 0.999	0.953	0.895 – 1.014
Tricipital skinfold (mm)	0.984	0.958 – 1.010	0.972	0.936 – 1.010
Transversal psoas muscle index (cm/m), Men: n=110, Women n=76	0.910	0.836 – 0.991	0.965	0.864 – 1.079
Myostatin baseline (per 100 pg/ml)	0.977	0.964 – 0.990	0.995	0.983 – 1.010
Etiology alcohol (%)	1.865	0.934 – 3.724	1.425	0.855 – 2.374
Sarcopenia (low HGS) (%)	1.703	1.100 – 2.630	1.574	0.920 – 2.690
Sarcopenia (low TPMI) (%) Men: n=110, Women n=76	1.801	0.995 – 3.257	1.145	0.515 – 2.552
Myostatin < cut-off (%) Men < 1600 pg/ml, Women < 2300 pg/ml	2.407	1.550 – 3.741	1.280	0.757 – 2.165
C-reactive protein > 10 mg/l	2.143	1.370 – 3.352	2.276	1.325 – 3.908
Acute-on-chronic liver failure (%)	4.004	2.110 – 7.610	3.377	1.734 – 6.578

take time and require additional software. In the present study, we explore the usefulness of serum myostatin as a simple marker of sarcopenia and adverse outcome of ACLD. We provide evidence that in men myostatin level directly reflects the total muscle mass and patients with myostatin < 1600 pg/ml had 1.83 times the likelihood of dying during follow-up regardless of the baseline MELD score or CRP. Consequently, in clinical practice men with low myostatin may be regarded as high-risk patients requiring more aggressive management. In addition, in cases with low myostatin, we also observed a trend towards the lower probability of liver transplantation. This finding probably reflects the fact that these patients had a more severe general condition and were less fit for the surgery.

The usefulness of serum myostatin in ACLD has been recently reported in a study by Nishikawa *et al*. in 198 patients (7). Median myostatin values were found to be higher when compared to our cohort (3419 in men and 2662 pg/ml in women). In this study, the authors showed an indirect correlation between myostatin and muscle mass and confirmed that higher than median myostatin levels were associated with poorer long-term survival. This finding is in contrast to the results of our study, which reports that patients with myostatin lower than the identified cut-off had significantly worse survival. This obvious difference appears to be reflecting the association between myostatin and muscle mass. Unlike in the study by Nishikawa *et al*. (7), we report a direct correlation between myostatin and MAC, HGS or TPMI. Moreover, higher myostatin values in the control group are also consistent with this observation. Some other studies have found a direct correlation between myostatin and muscle mass. In 69 outpatients with chronic kidney failure undergoing peritoneal dialysis, Yamada *et al*. (20) reported a direct correlation between myostatin and lean body mass. In another study among 74 obese out-patients in Japan, authors reported a direct association between myostatin and muscle mass (21). A very similar association has also been reported by Peng *et al*. (22) among 463 healthy community-living elderly in whom low myostatin levels were independent predictors of relative appendicular skeletal muscle mass. In contrast, some studies did report an indirect correlation. For example, in 71 patients with chronic obstructive pulmonary disease myostatin indirectly correlated with skeletal muscle mass in men (23). For the moment, it is unclear what is the reason behind these diverging reports, however profound differences among study populations may be explaining the results. First, our study included only cases with acute decompensation apparently having a more profound liver dysfunction with 199 cases being in the CPT class C compared with only 3 cases in the Nishikawa *et al*. study (7). This is also evident from the differences in reported survival rate that was 75.7 or 73.3% at 90 days compared with 93.9 or 96.0% at 1 year. Second, we found a strong negative correlation between myostatin and inflammatory status while the study by Nishikawa does not address this issue. Myostatin is a member of the TGF-β family of cytokines and in the current study, we show (*Figs*. *1-3*) that an inflammatory state could be lowering its levels in an acute

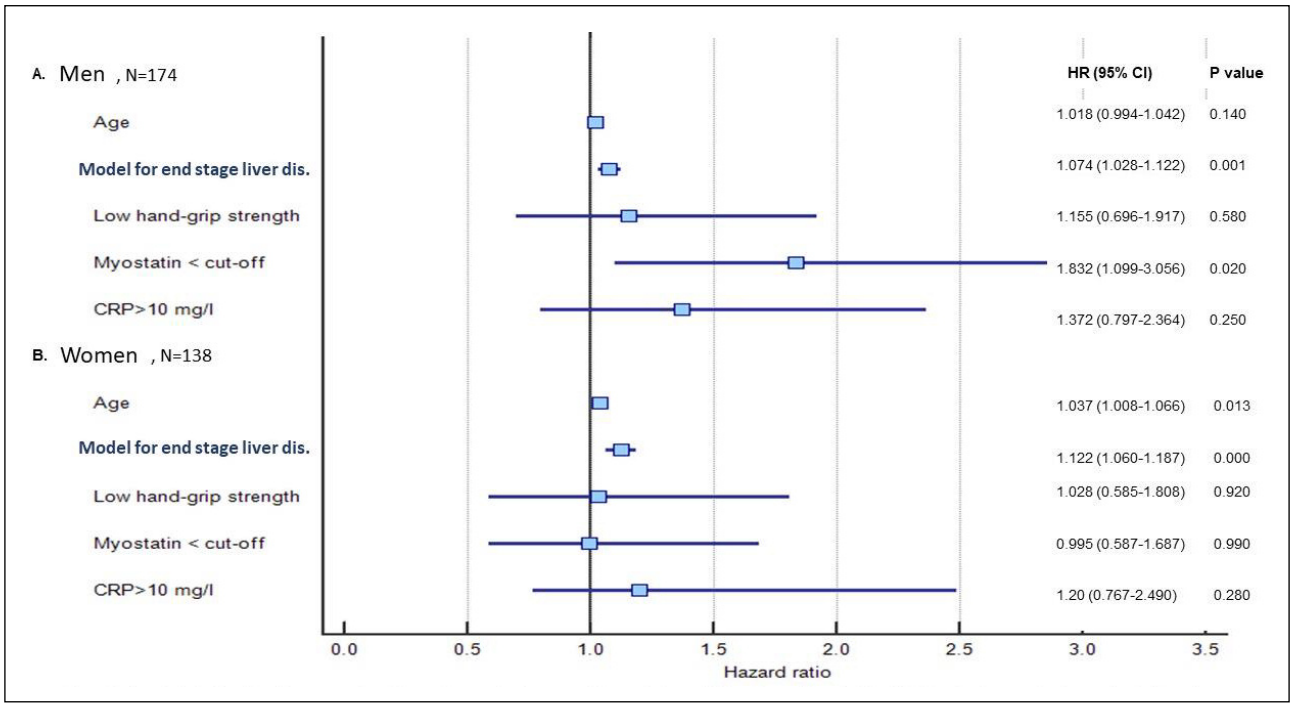

Fig. 8. Forest plot of the Fine Gray proportional hazard regression for competing events in prediction of overall survival in decompensated advanced chronic liver disease patients according to gender. A: men; B: women. Low hand grip strength: men < 30 kg, women < 20 kg. Myostain cut-off: men < 1600 pg/ml, women < 2300 pg/ml.

setting. Third, alcoholic liver disease and particularly active alcohol consumption could be an important confounding factor having an effect on muscle metabolism and myostatin concentration (24). In our study, almost 70% of cases had alcoholic liver disease, many of whom had active alcohol abuse a few days prior to baseline blood sampling. Patients with alcoholic liver disease had a trend towards the lowest median concentration of myostatin further confirming this association. In contrast, in the cohort of Nishikawa *et al*. (7) only 19 of 198 cases had alcoholic cirrhosis. Fourth, the follow-up time of our study was 400 days compared with 7 years in the latter study. Fifth, it has been reported that mortality related to sarcopenia is higher in Asian population than in Western ethnicities probably reflecting lower total muscle mass in healthy subjects and perhaps a slightly different pathogenesis (25).

Results of our study may also be regarded as being complementary to the previous reports. They appear consistent with the fact that myostatin is secreted by myocytes. In patients with a terminal disease and low muscle mass, there would be fewer functioning myocytes resulting in lower myostatin values and worse survival. We hypothesize, that as ACLD enters the stage of decompensation, there would be a decrease in myostatin levels. This observation could be explained by the systemic inflammation, immune system dysfunction, sepsis-induced myocyte impairment, elevated serum ammonia, lack of energy intake (26) or previous cumulative muscle loss. These mechanisms appear to be common among various conditions causing sarcopenia, but there is probably a unique combination of factors for any clinical disease or disease stage. Contrary to the previous reports showing that the decrease in myostatin was accompanied by improved muscle and bone regeneration (27), myostatin decrease in the present study was associated with worse overall survival. Thus, some pieces of this puzzle are yet missing from our understanding. Future studies on cause and effect of myostatin dynamics during prolonged inflammatory state, alcohol intake, and on its value in a pre-transplant setting in comparison to liver frailty index (28) or newer biomarkers (29) are warranted.

The present study provides evidence on the interference of inflammatory status with the relationship between myostatin and muscle parameters (*Figs*. *2* and *3*). Since a large proportion of patients had systemic inflammation at baseline, it is likely that it was the factor that contributed to lowered myostatin values as compared with the Nishikawa study. The relationship between myostatin values and muscle parameters in patients having ACLD associated inflammatory status has not been previously reported. However, in one study in 45 patients undergoing elective orthopedic surgery or coronary bypass myostatin has been measured prior to surgery on day 4 and on day 30. Elective surgery in these patients led to a significant drop in circulating myostatin on day 4 and 30 (30). This drop was accompanied by a significant increase in median CRP while the lowest myostatin concentration time point coincided with the highest CRP concentration time point. We might speculate that increased CRP might interfere with myostatin secretion from the muscle. One proposed mechanism of this phenomenon has been reported in an experimental model. Authors of this study have quantified myostatin signaling following sepsis induction in mice. They have observed a significant downregulation of myostatin mRNA levels in sepsis with no change in muscle myostatin (31). Our observation suggests that myostatin should be preferably measured once systemic inflammation has resolved, but it is yet unclear at which particular moment in time.

In women with dACLD who were explored in our study, some parameters of muscle mass and HGS did correlate with serum myostatin. However, unlike in men myostatin did not demonstrate any prognostic value. Lower prognostic implication of sarcopenia in women has been previously reported. In women, nutritional status evaluated by the Royal Free Hospital subjective global assessment has not been predictive of survival (4). In another study among community-dwelling older adults, serum myostatin

Table 5. Causes of death and myostatin concentration among 149 deceased patients during follow-up.

Causes of death	**Men** n = 85 myostatin median [IQR]			**Women** n = 64 myostatin median [IQR]		
Acute on chronic liver failure, hepatorenal syndrome	8	1267.6	262.7 – 2638.7	3	1267	817.1 – 2438.5
Portal hypertension bleeding	10	1447.6	1396.7 – 2190.6	5	2234.8	1495.8 – 2676.2
Vascular causes (myocardial infraction, pulmonary embolism, stroke)	3	1562	1359.5 – 3330.8	6	1402.4	1122.2 – 4864.8
Liver failure	7	1696.7	1292.0 – 2279.9	7	1051	682 –2056.4
Sepsis/infection	14	845.7	5364 – 4777.5	7	1013.8	916.3 – 2129.2
Unknown/lost to follow up	43	1498.7	821.1 – 2478.6	36	1921.3	1125.6 – 3267.8
P value		P = 0.948			P = 0.742	

was independently associated with sarcopenia in men, but not in women (32). Moreover, serum concentrations of TGF-β superfamily including myostatin did not reflect stages of sarcopenia in elderly women (33). A recent study among healthy older community-living men and women reports a very similar observation to our study. Unlike in men, low myostatin levels weakly correlated with muscle mass in women (22). This observation may be explained by different body composition. A recent study has reported that in women with cirrhosis, it is the low subcutaneous adiposity that is associated with higher mortality (34). Our study is consistent with this report since all muscle parameters were lower in women, while the tricipital skinfold reflecting subcutaneous fat was significantly higher. Consequently, the progression of cirrhosis may be causing gender-specific patterns of muscle and fat loss. Some authors have proposed that sarcopenia in women is driven by malnutrition, whereas in men it is presumably driven by hypoandrogenism (15). Another plausible explanation for these findings has been reported in athletes after eccentric muscle contractions causing muscle damage. Circulating estradiol has been reported interfering with the skeletal muscle myostatin mRNA expression in women, but not in men (35). To date, apart from different estrogen and DHEA signaling, there is little evidence for the explanation of the observed sex-related differences (22).

Our study has several strengths. It is the largest from the few studies evaluating the utility of myostatin levels in the management of the end-stage liver disease. It is the only study evaluating the usefulness of serum myostatin in the context of decompensated ACLD and is the first study to explore the relationship between myostatin and inflammatory status. Our study has a prospective design, large sample size, real-life setting with no selection bias only for transplant candidates and sufficient follow-up time.

Our study has some limitations. First, the study cohort included consecutive patients with ACLD regardless of their liver transplant status including patients with active alcohol abuse or other contraindications for liver transplantation. Therefore, the prognosis of these patients could also be influenced by factors different from liver disease and related sarcopenia. Second, we could measure the central muscle mass from CT scans only in 186 out of 355 cases as mentioned in the methods section. Furthermore, due to our CT scan software equipment, we could not measure the skeletal muscle index, which is currently considered the reference method for diagnosing sarcopenia in ACLD. However, there is evidence that measuring psoas muscle diameters could also be used to identify ACLD patients at risk for adverse outcomes (13, 14). Third, although the reported association between myostatin, muscle mass and survival is statistically significant, there is an inherent risk of statistical error. For example, AUROC values of 0.75 still carry a 25% chance of error. Fourth, our control group does not match in number, gender or age with the study group. We measured myostatin levels in the control group for the reason of assuring that the method is valid and the control group had values in the reference range that has been defined by the manufacturer of the test. Fifth, we could only provide causes of death in 70 out of 149 patients due to the fact, that patients have died in various regions around the country (*Table 5*).

In summary, the present study reports that the interpretation of serum myostatin in dACLD is complex with many factors influencing its levels. In men, myostatin values correlate with muscle mass, yet this association is hampered by the inflammatory status. The precise time-point for myostatin measurement in relation to acute cirrhosis complications remains disputable. Furthermore, in men, low myostatin levels (< 1600 pg/ml) could predict worse overall survival independently on MELD, CRP or muscle strength, identifying cases with worse prognosis and a lower likelihood of liver transplantation. In contrast, serum myostatin shows no evident clinical utility in women.

Conflict of interests: None declared.

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