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Research Article

Serum Follistatin as Short-Term Prognostic Markers for Patients with Chronic Liver Disease

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1. Abstract

1.1. Background: Chronic Liver Disease (CLD) is caused by several agents and leads to mortality through various mechanisms. Several prognostic factors have been discussed for CLD. Follistatin (FST) is a potential marker for liver function, muscle function, and metabolism. We evaluated whether FST correlated with prognosis and clinical factors.

1.2. Methods: A series of 185 patients with CLD who visited our hospital between May 2017 and June 2019 was enrolled in this study. The mean observation period was 358 days. Patients were evaluated for liver disease etiology, albumin-bilirubin index (ALBI), model for end-stage liver disease (MELD), and fibrosis-4 (FIB-4). Serum FST and activin A (ACT) were assayed using commercially available assay kits. Of the 185 patients, 150 underwent Computed Tomography (CT). Cross-sectional CT images of the third lumbar vertebrae were analyzed using commercial software to determine body composition.

1.3. Results: In the observation period, 14 patients died. In the dead and survivor groups, the observation period was 241 and 368 days, respectively. MELD, FIB-4, ALBI grade, ACT, and FST at admission differed between the dead and survivor groups and influenced the survival periods. In multivariate logistic regression analysis, high FST was the only factor associated with survival. In the body composition assay using CT, FST was associated with subcutaneous adipose tissue, muscle attenuation, and total bilirubin.

1.4. Conclusions: FST is a simple surrogate maker for prognosis in CLD. High FST is a potential aggregation marker of advanced liver damage, low muscle quality, and subcutaneous adipose tissue volume.

2. Abbreviations: eGFR: Estimated glomerular filtration rate; Cr: Creatinine; CysC: Cystatin C; SI: Sarcopenia index; CBMM: Calculated body muscle mass; HCV: Hepatitis C virus; CH: Chronic hepatitis; LC: Liver cirrhosis; TB: Total bilirubin; ALB: Albumin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PLT: Platelet; PT: Prothrombin time; MELD: Model for end-stage liver disease; FIB-4: Fibrosis-4; ALBI: Albumin-bilirubin score; GS: Grip strength; SM: Skeletal muscle; CT: Computed tomography; L3: Third lumbar vertebra; BMI: Body mass index; SMI: Skeletal muscle index; CBMMI: Calculated body muscle mass index; HU: Hounsfield unit

3. Keywords: Follistatin; Chronic liver disease; Prognosis, Subcutaneous adipose tissue, Muscle attenuation

4. Introduction

Chronic Liver Disease (CLD) is caused by several agents and leads to mortality through various mechanisms, including Hepatocellular Carcinoma (HC), which is a major cause of death, and liver failure [1]. Prognostic factors have been discussed for CLD. Model For End-Stage Liver Disease

(MELD), combined total bilirubin (TBIL), creatinine, and prothrombin time (international normalized rate) are established prognostic markers for liver disease [2]. The albumin-bilirubin (ALBI; combined albumin and total bilirubin) grade also offered a discriminatory method of assessing liver function [3]. Fibrosis-4 (FIB-4), combined platelets, alanine aminotransferase (ALT), Aspartate Aminotransferase (AST), and age have been evaluated as accurate markers of fibrosis [4]. These markers were calculated using liver-related factors before being recognized as liver-related prognostic factors.

Sarcopenia is a harmful condition in patients with liver disease and cirrhosis [5], those who undergo liver transplantations [6], and those with HCC [7]. Ammonia and other liver-related factors have been shown to exacerbate the worsening effects in skeletal muscle (SM) [8]. In addition to muscle volume loss, muscle quality (including fat deposition in muscle) is also an important prognostic factor for liver disease [7]. Muscle factors, including volume loss and fat deposition and the Barcelona Clinic liver Cancer stage, were independent predictors of HCC outcomes [7]. Therefore, liver and muscle factors should be evaluated for their association with survival in patients with CLD.

Follistatin (FST) is known as an inactivating factor for activin A (ACT) [9], bone-morphogenetic protein [10], and myostatin [11], and has bioactivities in muscle and cancers [12]. FST was identified as a glycoprotein that inhibits the synthesis and secretion of the follicle-stimulating hormone from the pituitary gland and is not a member of the TGF-β super family [13]. It has been reported that FST correlates with the fat-free muscle area in patients with cirrhosis [14]. FST was reported as a myokine at first [12] but is now known as a hepatokine [15]. Circulating FST is liver-derived and regulated by the glucagon-to-insulin ratio [15]. Patients with cirrhosis show an impaired capacity to acutely secrete FST, and a decrease in acute FST release may contribute to the loss of muscle mass in liver cirrhosis [16]. It has been reported that serum FST is significantly decreased in patients with HCV compared with controls [17]. However, FST levels were significantly elevated in patients with acute severe hepatitis and acute liver failure [18]. In contrast, elevated circulating FST levels are strongly associated with insulin resistance in patients with type 2 diabetes [19]. Serum FST was reduced in parallel with glycated hemoglobin in obese individuals with diabetes who underwent therapeutic gastric bypass surgery [20]. Hepatic FST is speculated to be the pathological hepatokine for diabetes. As a result, we speculate that FST may be a potential marker for liver function, muscle function, and metabolism.

In this study, we explored the prognostic factors for CLD and compared the survival periods with MELD, ALBI, FIB-4, and FST at the start of observation. Next, we evaluated whether FST correlated with clinical factors.

5. Methods

5.1. Patients

A series of 185 patients with CLD who visited Nagasaki Harbor Medical Center between May 2017 and Jun 2019 was enrolled in this study (Table 1). The mean observation period was 358 days (standard deviation (SD): 268 days). Patients were evaluated for the liver disease etiology (hepatitis C virus [HCV], hepatitis B virus [HBV], and others [N]), degree of liver damage (ALBI [3], MELD [2] and FIB-4 [4]), renal function (serum creatinine (Cr), cystatin C (CysC), Cr-based estimated glomerular filtration (Cr-GFR), and CysC-GFR), body mass index (BMI; BW [kg]/height2 [m2]), and grip strength (GS; kg). ALBI was classified from grades 1-3. The cutoff points were as follows: ≤-2.60 (ALBI grade 1), -2.60 to -1.39 (ALBI grade 2), and>-1.39 (ALBI grade 3).The maximum GS of the two tests was used for further analysis. Using the Japanese society of hepatology (JSH) criteria, female patients with a maximum GS <18 kg and male patients with a GS <26 kg were categorized as the low GS group [21].

Informed consent was obtained from each patient included in the study, and the patients were guaranteed the right to leave the study whenever they wished. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as evidenced by the approval of the study by the Human Research Ethics Committee of Nagasaki Harbor Medical Center (No. H30-031).

Table 1: Patient profiles of the dead and survivors.

	Dead(n=14)	Survivor(n=171)	p-value
Observation period	241.36 (300.54)	368.13 (264.8)	0.0899
Age	70.79 (14.8)	63.74 (15.27)	0.0977
Female/Male	6-Aug	92/79	0.9999
HBV/HCV/N	2/4/2008	30/69/72	0.091
PH of Malignancy All	3	27	0.7039
PH of HCC	1	18	0.9999
BW	49.73 (12.22)	61.08 (15.55)	0.0087
BMI	21.26 (3.42)	23.72 (4.96)	0.0816
Grip strength	12.83 (10.64)	20.63 (9.99)	0.0076
GS Low/within normal limited	3-Nov	93/78	0.097
AST	114.07 (171.61)	69.6 (164.27)	0.333
ALT	89.36 (192.13)	75.92 (211.81)	0.8185
TBIL	3.94 (7.13)	0.894 (0l887)	<0.0001
ALB	2.93 (0.831)	4.05 (0.574)	<0.0001
PT%	76.64 (19.17)	96.39 (18.15)	<0.0001
PTINR	1.196 (0.2)	1.045 (0.182)	0.0033
Platelet	15.02 (7.59)	20.14 (15.89)	0.2349
Cr	0.806 (0.28)	0.869 (0.8)	0.7727
Cr-eGFR	68.89 (24.48)	73.09 (24.29)	0.5357
CysC	1.79 (1.8)	1.17 (0.74)	0.01
CysC-eGFR	50.86 (23.55)	69.08 (27.99)	0.019
FIB-4	7.73 (5.64)	3.37 (3.69)	<0.0001
FIB-4 3.15H/L	3-Nov	54/117	0.0007
ALBI	-1.565 (0.871)	-2.719 (0.504)	<0.0001
ALBIG1/23	11-Mar	122/49	0.0003
MELD	11.65 (6.1)	7.83 (2.87)	<0.001
CKD12/345	6-Aug	127/44	0.2093
SI	58.11 (24.46)	73.9 (19.38)	0.0046
CBMM	28.66 (11.27)	35.64 (8.79)	0.0058
deGFR	18.03 (23.67)	4 (23.02)	0.0299
FST	2706.95 (1302.79)	1693.36 (1152.66)	0.002
ACT	1742.64 (2071.1)	703 (576)	<0.0001
FST/ACT ratio	2.58 (1.447)	2.869 (1.983)	0.5948

Numeric valuesare themean (standard deviation) or number. Observation period is in days. BW: body weight. BMI: body mass index. GS:grip strength. PH: past history. PH of malignancy included hepatocellular carcinoma (HCC, n=19), gynecological malignancy (n=3), colon cancer (n=2), pancreatic cancer (n=2), esophageal cancer (n=1), cholangiocellular cancer (n=1), gastric cancer (n=1), and breast cancer (n=1). Normal range of clinical parameters in fasting serum: aspartate aminotransferase (AST), 10-40 U/L;alanine aminotransferase (ALT), 5–40 U/L; total bilirubin (TBIL), 0.3-1.2 mg/dL; prothrombintime (PT), 70-130%, 0.85-1.15 [international normalized ra- tio(INR)]; albumin (ALB), 3.8–5.2 g/dL; platelets, male patients (M), 13.1–36.2 × 104/ μL; platelets, female patients (F), 13.0–36.9 × 104/μL; creatinine (Cr), M, 0.61-1.04 mg/dL; Cr,F, 0.47-0.79 mg/dL; cystatin C (CysC), M, 0.3-0.95 mg/L; CysC,F, 0.56-0.87 mg/L. All laboratory data measurements were taken after overnight fasting. Cr-based eGFR (Cr-eGFR), CysC-based eGFR (CysC-eGFR), FIB-4, ALBI, MELD, CKD, SI, CBMM, and deGFR were calculatedusingformulasdescribed in themethods. Follistatin (FST; pg/mL) and activin A (ACT; pg/mL) were measured by commercially available ELISA kits.

5.2. Laboratory Measurements

Laboratory data and anthropometric measurements were obtained for each subject during their hospital visit. Laboratory examinations included the assessment of platelet count; PT, Cr, CysC, albumin, total bilirubin, ALT, and AST.Cr- and CysC-eGFRs (mL/min/1.73 m2) in female and male patients were calculated using the Japanese Society of Nephrology for Japanese patients [22] guidelines. The difference (deGFR) was calculated as follows: Cr-based eGFRs – CysC-based eGFRs [23]. SI was calculated as follows: Cr/CysC × 100 [23, 24]. The calculated body muscle mass (CBMM) was calculated as follows: CBMM = (body weight (kg) × Cr)/ ([K × body weight (kg) × CysC) + Cr]) where K=0.00675 for men and K=0.01006 for women [23-25]. The CBMM index (CBMMI) was calculated as follows: CBMM/height2 (m2). Serum FST and ACT were assayed using commercially available assay kits (Human Follistatin Quantikine ELISA kit and Human Activin A Quantikine ELISA kit, R &D systems, Minneapolis, MN). The assay range was 250–16000 pg/mL for FST and 15.6–1000 pg/mL for ACT.

5.3. CT Analysis of Body Composition

Of the 185 patients, 150 were screened for HCC using Computed Tomography (CT). Cross-sectional CT images of the third lumbar vertebrae were analyzed using the Slice-O-Matic software (version 5.0; Tomovision, Montreal, Canada) to determine the SM mass. Muscle areas included the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques, and rectus abdominis muscles. Tissue Hounsfield unit (HU) thresholds were employed: -29–150 HU for SM; -190 to -30 for subcutaneous adipose tissue (SAT); and -150 to -50 for visceral adipose tissue (VAT) [26]. In addition, the mean muscle attenuation (MA) was calculated using the same CT images to assess SM quality (HU). SMs were normalized for height using m2 and expressed as cm2/m2 to determine the Skeletal Muscle Index (SMI). The low SMI group had SMIs <39 cm2/m2 for women, and <42 cm2/m2 for men. Sarcopenia was diagnosed as a low GS and SMI based on the JSH guidelines for sarcopenia [21].

5.4. Statistical Analysis

Data were analyzed using the Stat Flex 6.0 software (Artech Co., Ltd., Osaka, Japan) and are presented as the means ± standard deviations. Laboratory result variables were compared using correlation analyses, t-tests (for differences between two groups), and $\chi 2$ tests. Multi-regression analyses were performed and β was the standardized partial regression coefficient. Multivariate analyses were performed using logistic regression analyses. Correlations were evaluated based on Pearson's correlation coefficient (R). Receiver Operating Characteristic (ROC) curve analyses were used to evaluate the associations among groups and factors with the cutoff points being of equal value for sensitivity and specificity. Survival time analysis was evaluated by the Kaplan-Meier method and log-rank test. P-values <0.05 were considered statistically significant.

5.5. Results

Differences in clinical factors and body composition between the dead and survivor groups.

In the observation period, 14 patients died. In the dead and survivor groups, the observation period was 241 and 368 days, respectively. The causes of death were liver failure (6 patients, including 1 patient with liver transplantation), multiple-organ failure (2 patients), colon cancer (1patient), hepatocellular carcinoma (HCC) (1 patient), pancreatic cancer (1 patient), and unknown (2 patients). History of malignant disease included HCC (19 patients), gynecological cancers (3 patients), pancreatic cancer (2 patients), colon cancer (2 patients), cholangiocellular carcinoma (1patient), esophageal cancer (1 patient), gastric cancer (1 patient), and breast cancer (1 patient). Comparisons of the dead and survivor groups showed that body weight (BW), GS, TBIL, albumin (ALB), PT% and INR, CysC, CysC-eGFR, FIB-4, ALBI, MELD, SI, CBMM, deGFR, FST, and ACT were different (Table 1). However, the FST/ACT ratio did not differ between groups. Of the 185patients, 150 underwent body composition evaluation using CT at the start of observation (Table 2). SAT in the dead group (12 patients) was lower than that in the survivor group (138 patients). MA in the dead group tended to be lower than that in the survivor group, but other composition factors did not significantly differ between groups. Low SMI and the sarcopenia rate, defined by the JSH criteria, also did not significantly differ between groups.

MELD, FIB-4, ALBIG, ACT, and FST were related to survival in the observation period, but only FST contributed to survival.

Table 2: Body composition of the dead and survivors.

	Dead(n=12)	Survivor(n=138)	p-value
SM	98.6 (26.8)	109.77 (32.49)	0.2488
SMI	41.76 (6.93)	42.41 (9.9)	0.8295
IMAT	6.6 (7.52)	8.76 (7.87)	0.3567
VAT	67.59 (39.93)	105.09 (82.27)	0.121
SAT	79.58 (44.56)	133.05 (87.27)	0.0379
VSR	0.852 (0.52)	0.818 (0.598)	0.8471
MA	25.43 (8.72)	30.56 (8.19)	0.0587
SMI L/N	8-Mar	53/85	0.5367
Sarcopenia N/Y	3-Aug	114/38	0.9999

Body composition was evaluated using CT. Sarcopenia and SMI low/normal were defined according to the JSH criteria.
SM, IMAT, VAT and SAT were expressed as an area (cm2). MA is the mean HU in SM.
VSR is the VAT/SAT ratio.
L is Low, N is normal in SMI L/N.
N is not sarcopenia, Y is sarcopenia in Sarcopenia N/Y

To evaluate the cutoff point for death, ROC analysis was performed (Figure 1). Cutoff point for death was 1864 pg/mL in FST, 758 pg/mL in ACT, 10 in deGFR, 64.9 in SI, 30.72 in CBMM, 17.63 in GS, 3.76 in FIB-4, -2.5 in ALBI, and 7.47 in MELD. The area under the cure (AUC) for death was 0.81767 in FST, 0.7853 in ACT, 0.68424 in deGFR, 0.715633 in SI, 0.71261 in CBMM, 0.77068 in GS, 0.77068 in FIB-4, 0.82289 in ALBI, and 0.74696 in MELD. SAT and MA had cutoff values of 108.43 and 27.99, and an AUC of 0.70592 and 0.65474, respectively (Supplemental Figure 1). As a result of ROC analysis, we divided the patients into two groups. With respect to ALBI, patients were divided into G1 and G2/3 groups. The FIB-4 in FIB-4 high group >3.76. The MELD in MELD high group was

>7.47. Serum FST in FST high group was >1860 pg/mL. Serum ACT in ACT-high group was >758 pg/mL. The MELD-, FIB-4-, ALBIG2/3-, FST-, and ACT-high groups had a lower survival rate (Figure 2). In contrast, SI, deGFR, CBMM, SAT, and MA did not show significant differences between the high and low groups; and low GS and SMI, sarcopenia, and sex also did not significantly differ (Supplemental Figure 2). We evaluated death and survival factors (Table 3). High ALBIG1, FIB-4, MELD, FST, as well as ACT were found to contribute to death in the univariate analysis, but high FST was the only contributive factor determined by multivariate analysis.

SAT, MA and TBIL contributed to FST.

Lastly, we explored FST-related factors (Table 4). TBIL, ALB, PT%, CPS, MELD, FIB-4, ALBI, CysC, CysC-eGFR were shown to be related to FST (Table 4A). In multi-regression analysis, TBIL and ALB contributed to FST, but PT% and CysC-eGFR did not. We also compared FST and body compositions (Table 4B). SM and SAT were related to FST, but MA was not. MA differed between the survivor and dead groups (Table 1). In multi-regression analysis, SAT was a contributive factor for FST, and MA had a tendency to contribute but SM did not. We evaluated SAT, MA, TBIL, ALB, and FST in patients using CT (Table 4C). SAT, MA, and TBIL were contributive factors for FST.

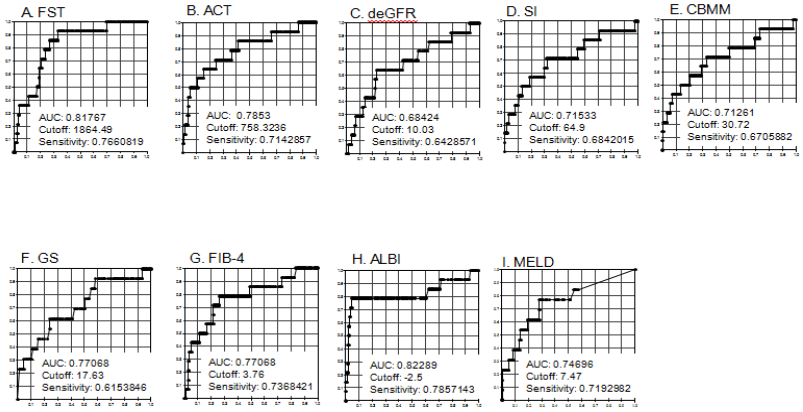
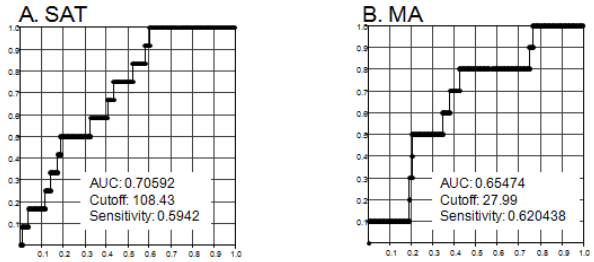


Figure 1: Receiver operating characteristic (ROC) curve analyses between death and clinical factors.The Y-axis is sensitivity and x-axis is 1-specificity.The area under the cure (AUC), cutoff (sensitivity =1-specificity), and sensitivity are described in each panel. A. FST; B. ACT; C. deGFR; D. SI; E. CBMM; F. GS; G.FIB-4; H. ALBI; and I. MELD.



Supplemental Figure 1: ROC curve analyses between SAT, MA, and death.
The Y-axis is the sensitivity and x-axis is the 1- specificity. The AUC, cutoff (sensitivity = 1-specificity), and sensitivity are described in each panel. A. SAT, B. MA.

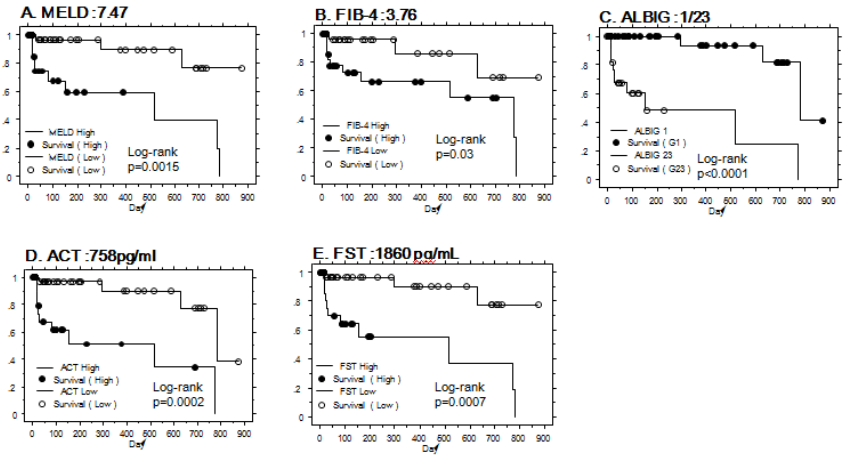
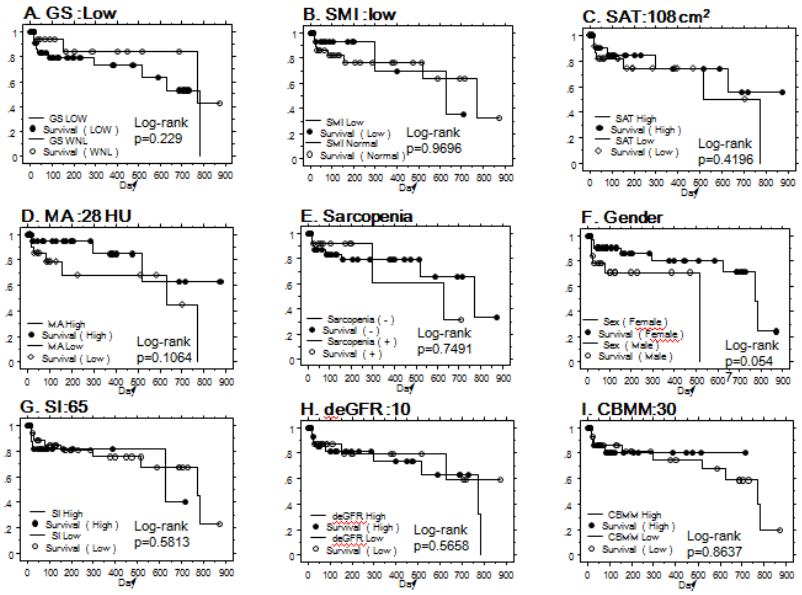


Figure 2: Survival curve and related factors.
The Y-axis is the survival rate and x-axis is the survival period (days). The survival line is determined using the Kaplan-Meier method with p-values (log-rank test) presented in each figure. A. MELD, High group is more than 7.47 in MELD. Low group is equal or less than 7.47. B.FIB-4. High group is more than 3.76 in FIB-4. Low group is equal or less than 3.76.C. ALBIG. G1 is grade 1 of ALBIG. G23 is grade 2 and 3.D. ACT. High group is more than 758 pg/mL in ACT. Low group is equal or less than 758 pg/mL. E.FST. High group is more than 1860 pg/mL in FST. Low group is equal or less than 1860 pg/mL.



Supplemental Figure 2: Survival curves and other factors.
The Y-axis is the survival rate and the x-axis is the survival period (days). P-values (log-rank test) are presented in each figure. A. GS: low and within normal groups are divided according to the JSH criteria. B. SMI: Low and within normal groups are divided according to the JSH criteria. C. SAT: High group is >108 cm2 of the SAT area. Low group is ≤108 cm2.D. MA: High group is > 28 HU. Low group is ≤28 HU. E. Sarcopenia: Sarcopenia (-) and (+) group is divided according to the JSH criteria. F. Sex: F is female and M is male.G. SI: High group is > 65. Low group is ≤65. H. deGFR: High group is >10. Low group is ≤10. I. CBMM: High group is > 30. Low group is ≤30.

Table 3: Analysis of contributive factors for death Univariate and multivariate analysis for death using logistic regression analysis.

	Univariate			Multivariate		
	p-value	odds	95% CI	p-value	odds	95% CI
ALBIG1	0.001	0.11	0.029-0.41	0.1858	0.348	0.073-1.662
FIB-4 High	0.002	7.944	2.129-29.645	0.1822	2.805	0.616-12.766
MELD High	0.0016	8.542	2.253-32.386	0.1928	2.931	0.581-14.786
FST High	0.0003	11.626	3.093-43.701	0.0053	9.946	1.979-49.99
ACT High	0.0022	6.596	1.972-22.058	0.9935	1.007	0.469-21.008
ALBIG1 is an ALBI grade of 1. FIB-4 High is >3.76. MELD High is >7.47. FST High is >1860pg/mL. ACT High is >758 pg/mL.						

6. Discussion

In this study, MELD, FIB-4, ALBIG, ACT, and FST at admission differed between the dead and survivor groups and influenced the survival periods. In multivariate logistic regression analysis, high FST was the only factor associated with survival. Ina CT-based assay of body composition, FST was found to be related to SAT, MA, and TBIL in multi-regression analysis.

Previously, it was reported that myostatin was associated with worse survival in patients with cirrhosis, and higher serum myostatin levels correlated with muscle mass loss [26]. However, age and psoas muscle index were contributive factors for overall survival, but serum myostatin did not have a significant influence for survival with or without HCC in multivariate analysis [26]. Meanwhile, FST levels gradually increased with the rise of TNM staging and category in lung cancers and may be associated with disease progression and metastasis [27]. FST increased in the circulation of patients with HCC and was expressed in hepatoma cells [28]. However, it had not been evaluated whether FST was related to prognosis in CLD.

It has been reported that fat-free muscle mass area is related to FST in 45 patients with decompensated cirrhosis [14] and serum FST was significantly decreased in 40 patients with chronic hepatitis C (CHC) [17]. Additionally, baseline FST levels did not differ between 8 healthy controls and 8 patients with cirrhosis [16]. However, patients with alcoholic liver disease and alcoholic cirrhosis have significantly higher FST [29]. High levels of serum FST adversely affected of sorafenib treatment in HCC patients [30]. Pathological increases of both ACT and FST by hepatocytes are associated with severe liver disease, including fibrosis, cirrhosis, and HCC [31]. Acute severe liver disease associated with high FST levels and a decreased FST/ACT (FA) ratio is an indicator of poor prognosis in acute liver failure [18]. In this study, high FST levels were associated with poor prognosis in CLD and TBIL. Since the cause of death in patients was HCC in this study, chronic liver damage should be considered the cause of high FST levels. Changes of FST in CHC were also evaluated for the relation to the phase of disease progression [31].

It has been reported that FST inhibits the action of myostatin [11.12] and is positively correlated with muscle volume [14]. However, in this study, FST was not related to CBMM and was weakly and negatively correlated to SM. Meanwhile, MA was associated with FST in this study. A low MA was equal to myosteatosis and independently associated with mortality in cirrhosis [32]. Additionally, a lower MA was associated with HCC development [33] and poor prognosis for HCC [7]. Since MA is also a prognostic factor in CLD, it makes sense that FST is associated with MA. Although it is not known if FST can cause myosteatosis, high FST levels are associated with type 2 diabetes [19] and insulin resistance [20]. Inter muscular adipose tissue has also been shown to directly modulate SM insulin sensitivity [34]. The relationship between FST, insulin resistance, and myosteatosis should be evaluated in the future as FST has been known to play a role in metabolic control.

Table 4: Comparison of clinical factors, body composition, and FST.
Correlations between FST and clinical factors were evaluated based on Pearson's correlation coefficient and multi-regression analysis was performed between FST and clinical factors.

A. Clinical factors (185 cases)				
	R	p-value	β	p-value
Age	0.133	0.0714		
Body weight	-0.107	0.147		
BMI	-0.119	0.1095		
Grip strength	-0.11	0.1399		
Platelet	-0.039	0.5945		
AST	0.101	0.176		
ALT	0.041	0.579		
TBIL	0.357	<0.0001	0.345	<0.0001
ALB	-0.259	0.0003	-0.167	0.0451
PT%	-0.145	0.0489	0.063	0.4209
PTINR	0.127	0.0861		
CPS	0.306	<0.0001		
MELD	0.303	<0.0001		
FIB-4	0.404	<0.0001		
ALBI	0.332	<0.0001		
Cr	0.082	0.2679		
Cr-eGFR	-0.087	0.2393		
CysC	0.157	0.0325		
CysC-eGFR	-0.171	0.0196	-0.099	0.189
SI	-0.108	0.143		
CBMM	-0.087	0.2426		
deGR	0.116	0.1168		
B. Body composition (150 cases)				
	R	p-value	β	p-value
SM	-0.175	0.0325	0.033	0.7537
SMI	-0.159	0.052		
IMAT	0.028	0.7335		
VAT	-0.077	0.3486		
SAT	-0.219	0.007	-0.218	0.0194
VS	0.099	0.2315		
MA	-0.157	0.0573	-0.19	0.0569
C. Clinical factors and body composition (150 cases)				
	β	p-value		
SAT	-0.158	0.0426		
MA	-0.159	0.0448		
TBIL	0.331	<0.0001		
ALB	-0.129	0.1172		

R is the correlation coefficient. b is the standardized partial regression coefficient.
A. FST related to clinical factors in all patients.
B. FST related to body composition in patients using CT.
C. FST contributed to SAT, MA, and TBIL in patients using CT.

High FST was associated with low SAT in this study, and low SAT is a known prognostic factor in patients with cirrhosis [35] and cancer [36]. Little is known about FST action in adipocytes. Myostatin induces fat loss [37] while FST promotes adipogenesis in vitro [38] and is associated with serum cholesterol, triglyceride, and blood pressure [39]. According to previous reports [19, 20, 39], high FST is a metabolic parameter, and conflicts with low SAT. FST resistance in SAT is agenda in CLD.

A limitation of this study is the small sample size (185 patients) and short observation time (mean 365 days). CLD included various grades of liver damage (chronic hepatitis and cirrhosis). There was also variation in the cause of death and liver disease. However, when we examined patients with CLD, it indicated that the evaluation of FST was a simple surrogate marker for prognosis. High FST has the potential to be an aggregation maker of advanced liver damage, low muscle quality, and SAT volume. Challenges for the future are to determine whether FST is related to the development of HCC and the cause of cancer-related deaths, since HCC and other cancers included high levels of FST.

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