

# Serum hyaluronic acid predicts protein-energy malnutrition in chronic hepatitis C

Hiroki Nishikawa (MD), Hirayuki Enomoto (MD, PhD)\*, Kazunori Yoh (MD), Yoshinori Iwata (MD, PhD), Kunihiro Hasegawa (MD), Chikage Nakano (MD), Ryo Takata (MD), Kyohei Kishino (MD), Yoshihiro Shimono (MD), Yoshiyuki Sakai (MD, PhD), Takashi Nishimura (MD, PhD), Nobuhiro Aizawa (MD, PhD), Naoto Ikeda (MD, PhD), Tomoyuki Takashima (MD, PhD), Akio Ishii (MD), Hiroko Iijima (MD, PhD), Shuhei Nishiguchi (MD, PhD)

## Abstract

Serum hyaluronic acid (HA) is a well-established marker of fibrosis in patients with chronic liver disease (CLD). However, the relationship between serum HA level and protein-energy malnutrition (PEM) in patients with CLD is an unknown. We aimed to examine the relationship between serum HA level and PEM in patients with chronic hepatitis C (CHC) compared with the relationships of other serum markers of fibrosis. A total of 298 CHC subjects were analyzed. We defined patients with serum albumin level of  $\leq 3.5$  g/dL and nonprotein respiratory quotient  $< 0.85$  using indirect calorimetry as having PEM. We investigated the effect of serum HA level on the presence of PEM. Receiver operating characteristic curve (ROC) analysis was performed for calculating the area under the ROC (AUROC) for serum HA level, platelet count, aspartate aminotransferase (AST) to platelet ratio index, FIB-4 index, AST to alanine aminotransferase ratio, and Forns index for the presence of PEM. The median serum HA level in this study was 148.0 ng/mL (range: 9.0–6340.0 ng/mL). In terms of the degree of liver function (chronic hepatitis, Child-Pugh A, B, and C), the analyzed patients were well stratified according to serum HA level (overall significance,  $P < 0.0001$ ). The median value (range) of serum HA level in patients with PEM ( $n = 61$ ) was 389.0 ng/mL (43.6–6340.0 ng/mL) and that in patients without PEM ( $n = 237$ ) was 103.0 ng/mL (9.0–783.0 ng/mL) ( $P < 0.0001$ ). Among 6 fibrosis markers, serum HA level yielded the highest AUROC with a level of 0.849 at an optimal cut-off value of 151.0 ng/mL (sensitivity 93.4%; specificity 62.0%;  $P < 0.0001$ ). In the multivariate analysis, serum HA level was found to be a significant prognostic factor related to the presence of PEM ( $P = 0.0001$ ).

In conclusion, serum HA level can be a useful predictor of PEM in patients with CHC.

**Abbreviations:** %C = substrate oxidation of carbohydrate, %F = substrate oxidation rates of fat, %P = substrate oxidation of protein, ALP = alkaline phosphatase, ALT = alanine aminotransferase, APRI = AST to platelet ratio index, AST = aspartate aminotransferase, AUROC = area under the ROC, CHC = chronic hepatitis C, CLD = chronic liver disease, FFA = free fatty acid, GGT = gamma glutamyl transpeptidase, HA = hyaluronic acid, hCRP = high-sensitivity C reactive protein, HCV = hepatitis C virus, LC = liver cirrhosis, npRQ = nonprotein respiratory quotient, PEM = protein-energy malnutrition, PT = prothrombin time, REE = resting energy expenditure, ROC = receiver operating characteristic curve, SD = standard deviation, UN = urinary excretion of nitrogen, VCO<sub>2</sub> = carbon dioxide production per minute, VO<sub>2</sub> = oxygen consumption per minute, WBC = white blood cell.

**Keywords:** chronic hepatitis C, hyaluronic acid, liver fibrosis, predictive factor, protein-energy malnutrition

Editor: Wenyu Lin.

In the past year, Shuhei Nishiguchi received financial support from Chugai Pharmaceutical, MSD, Daiippon Sumitomo Pharma, Ajinomoto Pharma, and Otsuka Pharmaceutical. The remaining authors declare that they have no conflicts of interest to disclose.

Division of Hepatobiliary and Pancreatic Disease, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan.

\*Correspondence: Hirayuki Enomoto, Department of Hepatobiliary and Pancreatic Disease, Department of Internal Medicine, Hyogo College of Medicine, 1-1, Mukogawacho, Nishinomiya, Hyogo 663-8501, Japan (email: enomoto@hyo-med.ac.jp).

Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.

This is an open access article distributed under the Creative Commons Attribution-No Derivatives License 4.0, which allows for redistribution, commercial and noncommercial, as long as it is passed along unchanged and in whole, with credit to the author.

Medicine (2016) 95:24(e3920)

Received: 13 November 2015 / Received in final form: 29 April 2016 / Accepted: 18 May 2016

Published online 1 May 2016

<http://dx.doi.org/10.1097/MD.0000000000003920>

## 1. Introduction

The liver is an essential organ for the metabolism of 3 major nutrients: protein, fat, and carbohydrate.<sup>[1–5]</sup> Liver cirrhosis (LC) is often complicated with protein-energy malnutrition (PEM).<sup>[1,2,6–8]</sup> PEM can be assessed by measuring the serum albumin level for analyzing the degree of protein malnutrition, and by measuring the nonprotein respiratory quotient (npRQ) by using indirect calorimetry for assessing the degree of energy malnutrition; PEM is traditionally defined by observations of body composition (e.g., muscle mass and other anthropometric measurements, body weight, history of poor intake, and/or weight loss).<sup>[2,9,10]</sup> RQs reflect what macronutrients are being metabolized; values that approach 1.00 suggest that carbohydrates are largely being burned and values that approach 0.7 suggest that lipids are being consumed.<sup>[10,11]</sup> Patients with LC often have lower RQs, a phenomenon that has been attributed to limited stores of carbohydrates (e.g., glycogen).<sup>[10,11]</sup> In general, patients with a serum albumin level  $\leq 3.5$  g/dL and npRQ value  $< 0.85$  are considered to have PEM.<sup>[10]</sup> PEM is 1 of the most common complications seen in patients with LC.<sup>[1,2,6]</sup> PEM is linked to high morbidity and mortality in patients with LC and it

has recently attracted much attention as it is closely associated with sarcopenia.<sup>[1,2,4,6,7,11–13]</sup> Identifying patients with PEM is thus essential for ameliorating prognosis in chronic liver disease (CLD) with PEM.

On the other hand, due to the limitations of liver biopsy, such as small size of biopsy specimens or its invasiveness for evaluating the degree of liver fibrosis, various noninvasive tests have been used to assess the liver fibrosis stage.<sup>[14–17]</sup> In addition to various imaging modalities including fibroscan and acoustic radiation force impulse, there are various serum markers proposed for this purpose and 1 well-known serum marker is hyaluronic acid (HA).<sup>[14,18,19]</sup> HA is a high-molecular weight polysaccharide that is distributed in all body tissues and fluids.<sup>[18,20]</sup> HA is a component of the extra cellular matrix.<sup>[21]</sup> The liver is the essential organ involved in the degradation and synthesis of HA.<sup>[18,19]</sup> In the liver, HA is synthesized by Ito cells and finally degraded by sinusoidal endothelial cells.<sup>[22]</sup> In general, the serum HA level in patients with LC increases due to the decreased clearance of HA, which is related to the destruction of hepatocytes.<sup>[18]</sup> The usefulness of HA for predicting the degree of liver fibrosis has been well accepted in CLDs with different etiologies such as chronic hepatitis B or C, alcoholic liver disease, and nonalcoholic steatohepatitis.<sup>[18,23–26]</sup> This biomarker is worth assessing since it is reliable, easy, inexpensive, and freely available to measure.

Physicians and patients prefer to avoid a liver biopsy for fear of complications and evaluate the degree of liver fibrosis noninvasively. As mentioned above, many previous studies have demonstrated that HA is a useful marker for assessing the degree of liver fibrosis and it has been frequently used by some researchers to assess stages of liver fibrosis.<sup>[18–20,23–30]</sup> However, to the best of our knowledge, there have been no studies investigating the relationship between serum HA level and PEM in patients with chronic hepatitis C (CHC). Thus the current study aimed to examine the relationship between serum HA level and PEM in patients with CHC compared with the relationships of other serum fibrotic markers.

## 2. Patients and methods

### 2.1. Patients

Between October 2005 and July 2012, nutritional evaluation using indirect calorimetry was performed in a total of 298 patients with CHC at the Division of Hepatobiliary and Pancreatic Disease, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan. In our hospital, nutritional evaluation using indirect calorimetry had been performed on an inpatient basis as a rule. In patients who agreed to be subject to nutritional evaluation using indirect calorimetry, it was routinely conducted in our department. All patients analyzed had detectable HCV-RNA and hepatitis B surface antigen negativity and in all of them, there was no clear evidence of drug-induced or alcoholic liver disease or of severe comorbid diseases such as nephrotic syndrome or severe systemic inflammatory disease that can affect the interpretation of our current data. We defined patients with serum albumin level of  $\leq 3.5$  g/dL and npRQ  $< 0.85$  as those with PEM, according to previous reports.<sup>[31,32]</sup> We prospectively collected clinical data for these patients and retrospectively investigated the effect of serum HA level on the presence of PEM in our cohort by comparing with other fibrosis markers including platelet count, aspartate aminotransferase (AST) to platelet ratio index (APRI), FIB-4 index, AST to alanine

aminotransferase (ALT) ratio, and Forns index. In addition, in patients with available stored sera, we performed further analyses using these stored sera (described later).

Serum HA level was measured by using a particle-enhanced turbidimetric immunoassay.<sup>[18,19]</sup> The APRI score was calculated using Wai formula: (AST/upper limit of normal)/platelet count (expressed as platelets  $\times 10^9/L$ )  $\times 100$ .<sup>[33]</sup> The FIB-4 index was calculated using Sterling formula as: age (years)  $\times$  AST (IU/L)/platelet count ( $\times 10^9/L$ )  $\times \sqrt{ALT}$  (IU/L).<sup>[34]</sup> The Forns index was calculated as reported previously.<sup>[35]</sup>

Liver biopsy specimens were obtained using standard methods, and well-experienced pathologists in our hospital evaluated the samples. Fibrosis stages were evaluated according to the METAVIR scoring system and the staging was performed on a degree of F0–F4 (F0—no fibrosis; F1—portal fibrosis without septa; F2—portal fibrosis with rare septa; F3—numerous septa without cirrhosis; F4—LC).<sup>[36,37]</sup> The pathological findings of the liver biopsy specimens were also routinely assessed in our department. We participated in conferences on the histological findings and final agreements were obtained.<sup>[37]</sup> In patients with poor liver function, after a full explanation of liver biopsy-related adverse events, we routinely used a thinner biopsy needle with great caution so as to avoid biopsy-related bleeding. All analyzed patients had no or minimal ascites on radiologic findings. In performing liver biopsy, procedure-related death was not observed in any of the analyzed cases.

The ethics committee of our hospital approved the current study protocol and this study protocol complied with all of the provisions of the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to liver biopsy and assessing nutritional status using indirect calorimetry.

### 2.2. Indirect calorimetry

Two parameters are measured using indirect calorimetry: carbon dioxide production per minute ( $V_{CO_2}$ ) and oxygen consumption per minute ( $V_{O_2}$ ). Total urinary excretion of nitrogen (UN) was measured as reported previously.<sup>[31,38]</sup> npRQ, resting energy expenditure (REE), substrate oxidation rates of fat (%F), carbohydrate (%C), and protein (%P) were calculated using the following formulas: npRQ =  $(1.44 V_{CO_2} - 4.890 UN)/(1.44 V_{O_2} - 6.04 UN)$ ; REE (kcal/d) =  $5.50 V_{O_2} + 1.76 V_{CO_2} - 1.99 UN$ ; F (g/24 h) =  $2.432 V_{O_2} + 2.432 V_{CO_2} - 1.943 UN$ ; C (g/24 h) =  $5.926 V_{O_2} + 4.189 V_{CO_2} - 2.539 UN$ ; P (g/24 h) =  $6.250 UN$ ; %F =  $9.46F/REE \times 100$ ; %C =  $4.18C/REE \times 100$ ; and %P =  $4.32P/REE \times 100$ .<sup>[31,38–41]</sup> Data for REE were obtained for all subjects in the morning after an overnight fast (12 h).

### 2.3. Statistical analysis

Receiver operating characteristic curve (ROC) analysis was performed for calculating the area under the ROC (AUROC) for serum HA level, platelet count, APRI, FIB-4 index, AST to ALT ratio, and Forns index by selecting the optimal cut-off value that maximized the sum of sensitivity and specificity for the presence of PEM. For continuous variables, the statistical analysis among groups was performed using Student *t* test, Mann–Whitney *U* test, Kruskal–Wallis test, or Spearman rank correlation coefficient  $r_s$  test as appropriate. For categorical variables, the groups were compared using Fisher exact test. Variables with  $P < 0.05$  in the univariate analysis were subjected to a multivariate logistic regression analysis. Data are expressed as means  $\pm$  standard deviation (SD) or median values (range). Values of  $P < 0.05$  were

**Table 1****Baseline characteristics in the current analysis (n=298).**

Variables	N=298
Age, y	64.0±12.0
Gender, male/female	147/151
Grade of histological fibrosis, F0/1/2/3/4	2/60/30/42/164
HCV-RNA ≥5 log copies/mL, yes/no	236/62
AST, IU/L	51.4±29.7
ALT, IU/L	47.2±32.1
ALP, IU/L	293.1±146.4
GGT, IU/L	54.1±63.9
Total cholesterol, mg/dL	157.5±39.4
Triglyceride, mg/dL	93.8±45.7
Serum albumin, g/dL	3.7±0.5
Total bilirubin, mg/dL	1.01±0.65
White blood cell, per mm <sup>3</sup>	4186±1580
Lymphocyte count, per mm <sup>3</sup>	1404±715
Platelet count, ×10 <sup>4</sup> /mm <sup>3</sup>	12.8±6.9
Prothrombin time, %	84.5±14.8
Serum hyaluronic acid, ng/mL	261.4±507.5
Body mass index, kg/m <sup>2</sup>	22.2±3.6
REE, kcal/d	1272.3±301.5
npRQ	0.88±0.10

Data are expressed as number or mean±standard deviation. ALP=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartate aminotransferase, GGT=gamma glutamyl transpeptidase, HCV=hepatitis C virus, npRQ=nonprotein respiratory quotient, REE=rest energy expenditure.

considered to be statistically significant. Statistical analysis was performed with the JMP 11 (SAS Institute Inc., Cary, NC).

### 3. Results

#### 3.1. Baseline characteristics

The baseline characteristics of the study participants (n=298) are shown in Table 1. They included 147 males and 151 females. The mean (±SD) age was 64.0±12.0 years. In terms of degree of liver fibrosis, there are 2 subjects with F0, 60 with F1, 30 with F2, 42 with F3, and 164 with F4. Patients with F4 included 97 with Child-Pugh A, 57 with Child-Pugh B, and 10 with Child-Pugh C. The mean (±SD) serum HA level was 261.4±507.5 ng/mL (median value; 148.0 ng/mL, range; 9.0–6340.0 ng/mL). In this analysis, 236 patients (79.2%) had HCV-RNA ≥5 log copies/mL.

#### 3.2. Prevalence of PEM in different fibrosis stages (F0–1, F2, F3, and F4) and different Child-Pugh stages (A, B, and C).

The proportions of PEM in different fibrosis stages were 1.6% (1/62) in F0–1, 6.7% (2/30) in F2, 4.8% (2/42) in F3, and 34.1% (56/164) in F4 (overall significance,  $P < 0.0001$ ; Fig. 1A). The proportions of PEM in different Child-Pugh stages were 16.5% (16/97) in Child-Pugh A, 52.6% (30/57) in Child-Pugh B, and 100% (10/10) in Child-Pugh C (overall significance,  $P < 0.0001$ ; Fig. 1B).

#### 3.3. Serum HA levels among patients with different degrees of liver damage

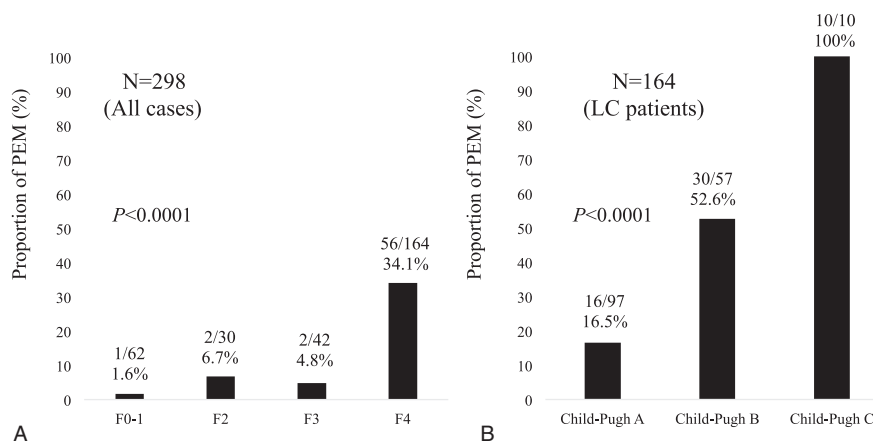
The median values (range) of serum HA levels among patients with different degrees of liver damage were as follows: 62.5 ng/mL (9.0–568.0 ng/mL) in patients with chronic hepatitis (F0–F3, n=134), 203.0 ng/mL (22.0–1290.0 ng/mL) in patients with Child-Pugh A (n=97), 358.0 ng/mL (55.8–3730 ng/mL) in patients with Child-Pugh B (n=57), and 775.0 ng/mL (165.0–6340.0 ng/mL) in patients with Child-Pugh C (n=10) (overall significance,  $P < 0.0001$ ; Fig. 2A).

#### 3.4. Comparison of serum HA level between patients with serum albumin value of >3.5 g/dL and those with serum albumin value of ≤3.5 g/dL

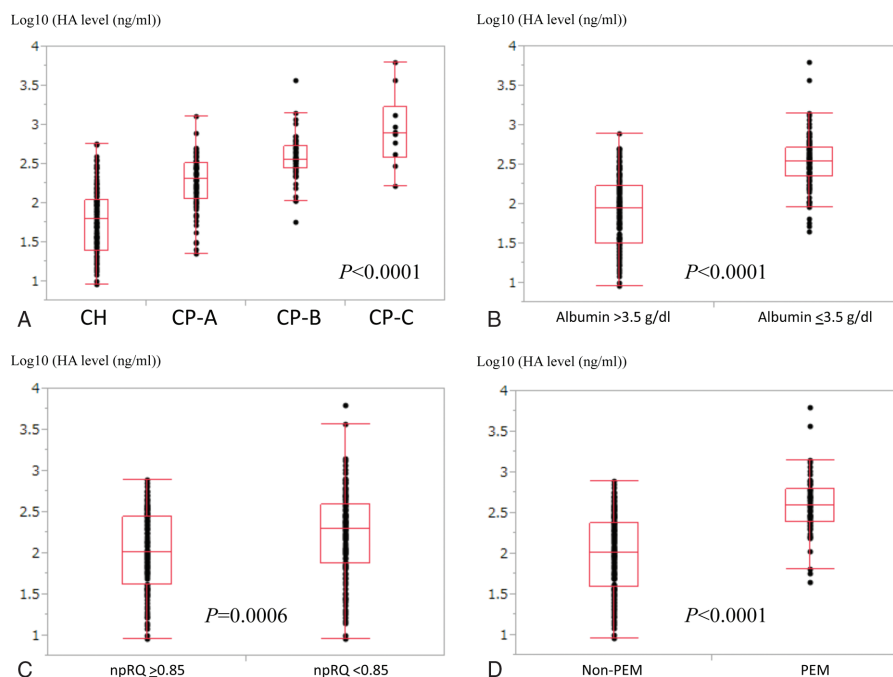
The median value (range) of serum HA level in patients with serum albumin value of ≤3.5 g/dL (n=104) was 346.0 ng/mL (43.6–6340.0 ng/mL) and that in patients with serum albumin value of >3.5 g/dL (n=194) was 87.8 ng/mL (9.0–783.0 ng/mL) ( $P < 0.0001$ ; Fig. 2B).

#### 3.5. Comparison of serum HA level between patients with npRQ value of ≥0.85 and those with npRQ of <0.85

The median value (range) of serum HA level in patients with npRQ value of <0.85 (n=141) was 199.0 ng/mL (9.0–6340.0 ng/mL) and that in patients with serum albumin value of ≥0.85 (n=157) was 102.0 ng/mL (9.0–783.0 ng/mL) ( $P = 0.0006$ ; Fig. 2C).



**Figure 1.** Prevalence of PEM in different fibrosis stages (F0–1, F2, F3, and F4) and different Child-Pugh stages (A, B, and C). (A) The proportions of PEM in different fibrosis stages were 1.6% (1/62) in F0–1, 6.7% (2/30) in F2, 4.8% (2/42) in F3, and 34.1% (56/164) in F4 (overall significance,  $P < 0.0001$ ). (B) The proportions of PEM in different Child-Pugh stages were 16.5% (16/97) in Child-Pugh A, 52.6% (30/57) in Child-Pugh B, and 100% (10/10) in Child-Pugh C (overall significance,  $P < 0.0001$ ). PEM=protein-energy malnutrition.



**Figure 2.** (A) Serum HA levels among patients with different degree of liver damage. The median values (range) of serum HA levels among patients with different degree of liver damage are 62.5 ng/mL (9.0–568.0 ng/mL) in patients with chronic hepatitis (CH [F0–F3], n = 134), 203.0 ng/mL (22.0–1290.0 ng/mL) in patients with Child-Pugh A (CP-A, n = 97), 358.0 ng/mL (55.8–3730.0 ng/mL) in patients with Child-Pugh B (CP-B, n = 57), and 775.0 ng/mL (165.0–6340.0 ng/mL) in patients with Child-Pugh C (CP-C, n = 10) (overall significance,  $P < 0.0001$ ). (B) Comparison of serum HA level between patients with serum albumin value of  $>3.5$  g/dL and those with serum albumin value of  $\leq 3.5$  g/dL. The median value (range) of serum HA level in patients with serum albumin value of  $\leq 3.5$  g/dL (n = 104) was 346.0 ng/mL (43.6–6340.0 ng/mL) and that in patients with serum albumin value of  $>3.5$  g/dL (n = 194) was 87.8 ng/mL (9.0–783.0 ng/mL) ( $P < 0.0001$ ). (C) Comparison of serum HA level between patients with npRQ value of  $\geq 0.85$  and those with npRQ of  $< 0.85$ . The median value (range) of serum HA level in patients with npRQ value of  $< 0.85$  (n = 141) was 199.0 ng/mL (9.0–6340.0 ng/mL) and that in patients with serum albumin value of  $\geq 0.85$  (n = 157) was 102.0 ng/mL (9.0–783.0 ng/mL) ( $P = 0.0006$ ). (D) Comparison of serum HA level between patients with and without PEM. The median value (range) of serum HA level in patients with PEM (n = 61) was 389.0 ng/mL (43.6–6340.0 ng/mL) and that in patients without PEM (n = 237) was 103.0 ng/mL (9.0–783.0 ng/mL) ( $P < 0.0001$ ). HA = hyaluronic acid, npRQ = nonprotein respiratory quotient, PEM = protein-energy malnutrition.

### 3.6. Comparison of serum HA levels between patients with and without PEM

The median value (range) of serum HA level in patients with PEM (n = 61) was 389.0 ng/mL (43.6–6340.0 ng/mL) and that in patients without PEM (n = 237) was 103.0 ng/mL (9.0–783.0 ng/mL) ( $P < 0.0001$ ; Fig. 2D).

### 3.7. ROC analyses of 6 fibrosis markers for the presence of PEM

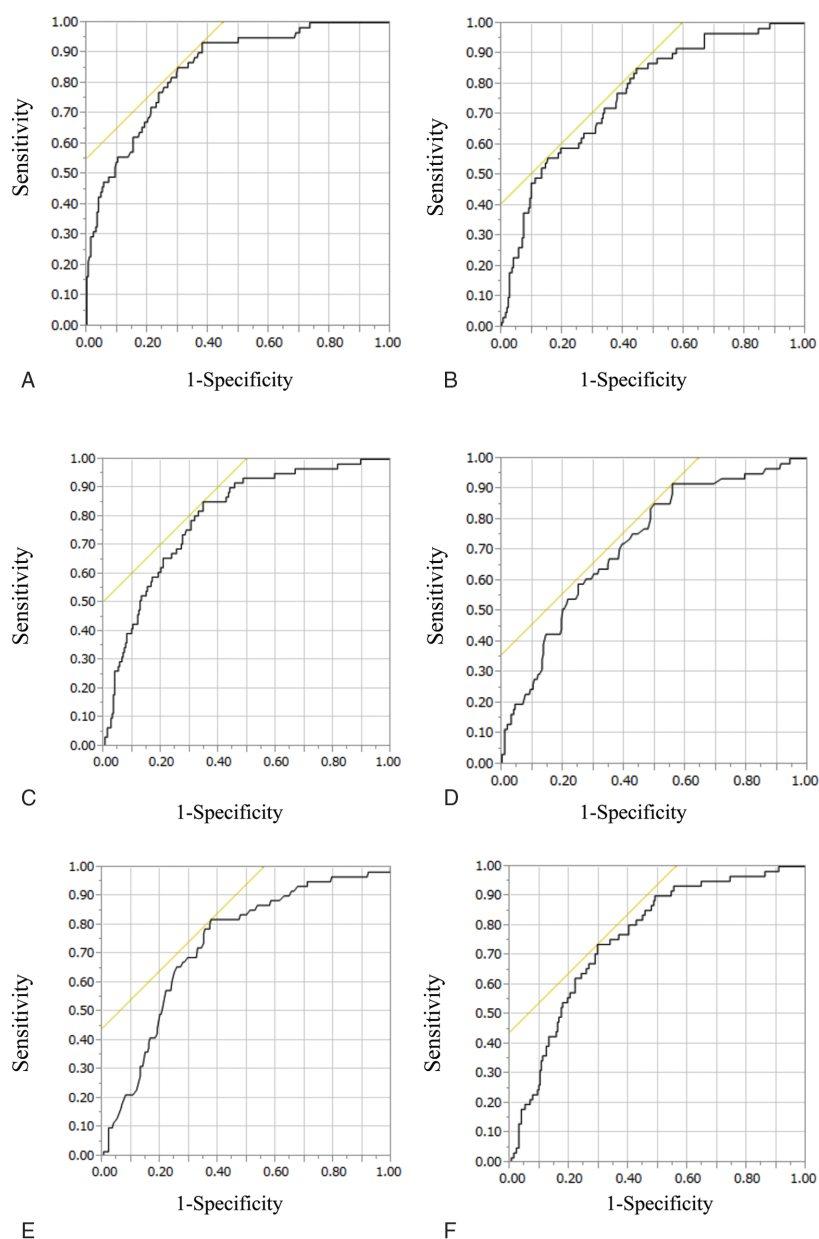
Serum HA level yielded the highest AUROC, with a level of 0.849, at an optimal cut-off value of 151.0 ng/mL (sensitivity, 93.4%; specificity, 62.0%;  $P < 0.0001$ ), followed by FIB-4 index (AUROC, 0.802;  $P < 0.0001$ ), APRI (AUROC, 0.770;  $P < 0.0001$ ), Forns index (AUROC, 0.762;  $P < 0.0001$ ), platelet count (AUROC, 0.734;  $P < 0.0001$ ), and AST to ALT ratio (AUROC, 0.724;  $P < 0.0001$ ; Fig. 3 and Table 2A). In patients with a serum HA level of  $\geq 151.0$  ng/mL (n = 147), the proportion of PEM was 38.8% (57/147), whereas in patients with a serum HA level of  $< 151.0$  ng/mL (n = 151), the proportion of PEM was 2.65% (4/151) ( $P < 0.0001$ ). When cut-off points of serum HA level were set at 300, 500, and 700 ng/mL, the proportions of PEM were 48.7% (38/78) in patients with HA level  $\geq 300$  ng/mL, 73.3% (22/30) in patients with HA level  $\geq 500$  ng/mL, and 87.5% (14/16) in patients with HA level  $\geq 700$  ng/mL. While in limited patients with LC (F4, n = 164), serum HA level also yielded the highest AUROC with a level of 0.771 at an optimal cut-off value of 443.0 ng/mL (sensitivity, 51.8%; specificity, 89.8%;  $P <$

0.0001; Table 2B). In patients with a serum HA level of  $\geq 443.0$  ng/mL (n = 40), the proportion of PEM was 72.5% (29/40), whereas in patients with a serum HA level of  $< 443.0$  ng/mL (n = 124), the proportion of PEM was 21.8% (27/124) ( $P < 0.0001$ ). In limited patients with non-LC (n = 134), APRI yielded the highest AUROC (0.854) and AUROC of serum HA level was 0.760 at an optimal cut-off value of 199.0 ng/mL (sensitivity, 60.0%; specificity, 91.5%;  $P < 0.0001$ ; Table 2C). In patients with a serum HA level of  $\geq 199.0$  ng/mL (n = 14), the proportion of PEM was 21.4% (3/14), whereas in patients with a serum HA level of  $< 199.0$  ng/mL (n = 120), the proportion of PEM was 1.7% (2/120) ( $P = 0.0082$ ).

### 3.8. Variables closely associated with HA value

Based on our results, we further investigated the relationship between HA value and other baseline variables by using Spearman rank correlation coefficient  $r_s$  test. In inflammatory diseases, the HA level is reported to be enhanced and free fatty acid (FFA) level is reported to be linked to npRQ value.<sup>[21,41,42]</sup> Thus we additionally tested high-sensitivity C reactive protein (hsCRP) and FFA level using stored sera. In this study, stored sera were available for 230 patients (77.2%).

For all cases, the variables significantly correlated with the HA value were as follows: age, white blood cell (WBC), lymphocyte count, AST, ALT, alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total bilirubin, serum albumin, platelet count, prothrombin time (PT), total cholesterol, triglyceride,



**Figure 3.** Receiver operating curve analyses of 6 fibrosis markers for the presence of PEM. (A) Serum hyaluronic acid level, (B) AST to platelet ratio index, (C) FIB-4 index, (D) AST to ALT ratio, (E) platelet count, and (F) Forns index. ALT=alanine aminotransferase, AST=aspartate aminotransferase, PEM=protein-energy malnutrition.

REE/body weight, body mass index, hCRP, and FFA. The  $r_s$  and  $P$  values for these variables are detailed in Table 3. For patients with LC ( $n=164$ ), the variables significantly correlated with the HA value were as follows: WBC, lymphocyte count, AST, ALP, GGT, total bilirubin, serum albumin, platelet count, PT, total cholesterol, triglyceride, hCRP, and FFA. The  $r_s$  and  $P$  values for these variables are detailed in Table 3.

### 3.9. ROC analyses of 6 fibrosis markers for the presence of PEM in limited patients whose stored sera were available ( $n=230$ )

In patients whose stored sera were available ( $n=230$ ), among the 6 fibrotic markers, serum HA level yielded the highest AUROC with a level of 0.848 ( $P<0.0001$ ), followed by FIB-4 index

(AUROC, 0.797;  $P<0.0001$ ), APRI (AUROC, 0.774;  $P<0.0001$ ), Forns index (AUROC, 0.764;  $P<0.0001$ ), platelet count (AUROC, 0.730;  $P=0.0001$ ), and AST to ALT ratio (AUROC, 0.719;  $P=0.0001$ ).

### 3.10. Univariate and multivariate analyses of factors linked to PEM for all cases

Univariate analysis identified the following factors as significantly associated with the presence of PEM: age ( $P=0.0009$ ); AST ( $P<0.0001$ ); ALP ( $P<0.0001$ ); total cholesterol ( $P<0.0001$ ); triglyceride ( $P=0.0043$ ); total bilirubin ( $P<0.0001$ ); WBC ( $P=0.0006$ ); lymphocyte count ( $P=0.0003$ ); platelet count ( $P<0.0001$ ); PT ( $P<0.0001$ ); serum HA level ( $P<0.0001$ ); APRI ( $P<0.0001$ ); FIB-4 index ( $P<0.0001$ ); AST to ALT ratio

**Table 2**

**Receiver operating curve (ROC) analyses of 6 fibrosis markers for the presence of PEM for all patients (A, n=298), patients with LC (B, n=164), and patients with non-LC (C, n=134).**

<b>(A)</b>					
<b>All patients (n=298)</b>	<b>AUROC</b>	<b>Cut-off value</b>	<b>P value</b>	<b>Sensitivity, %</b>	<b>Specificity, %</b>
HA level	0.849	151.0	<0.0001	93.4	62.0
APRI	0.770	1.27	<0.0001	85.2	55.7
FIB-4 index	0.802	4.88	<0.0001	85.3	65.4
AST to ALT ratio	0.724	1.0	<0.0001	91.8	44.3
Platelet count	0.734	10.7	<0.0001	82.0	62.5
Forns index	0.762	16.1	<0.0001	73.8	70.5
<b>(B)</b>					
<b>Patients with LC (n=164)</b>	<b>AUROC</b>	<b>Cut-off value</b>	<b>P value</b>	<b>Sensitivity, %</b>	<b>Specificity, %</b>
HA level	0.771	443.0	<0.0001	51.8	89.8
APRI	0.649	2.82	0.0037	48.2	81.5
FIB-4 index	0.670	8.16	0.0023	57.1	71.3
AST to ALT ratio	0.670	1.2	0.0006	76.8	50.9
Platelet count	0.592	10.7	0.1506	83.9	38.9
Forns index	0.626	16.8	0.0146	66.1	57.4
<b>(C)</b>					
<b>Patients with Non-LC (n=134)</b>	<b>AUROC</b>	<b>Cut-off value</b>	<b>P value</b>	<b>Sensitivity, %</b>	<b>Specificity, %</b>
HA level	0.760	199.0	<0.0001	60.0	91.5
APRI	0.854	1.47	<0.0001	80.0	82.2
FIB-4 index	0.851	7.06	<0.0001	60.0	99.2
AST to ALT ratio	0.596	1.08	0.011	80.0	61.2
Platelet count	0.765	8.9	<0.0001	60.0	89.15
Forns index	0.817	15.7	0.011	80.0	83.7

ALT = alanine aminotransferase, APRI = AST to platelet ratio index, AST = aspartate aminotransferase, AUROC = area under the ROC, HA = hyaluronic acid, LC = liver cirrhosis, PEM = protein-energy malnutrition

( $P < 0.0001$ ); Forns index ( $P < 0.0001$ ); and BMI ( $P = 0.0456$ ) (Table 4). The hazard ratios and 95% confidence intervals calculated using multivariate analysis for the 16 factors with  $P < 0.05$  in the univariate analysis are shown in Table 4. Serum HA level ( $P = 0.0001$ ) and PT ( $P = 0.0351$ ) were found to be significant prognostic factors related to the presence of PEM.

#### 4. Discussion

HA is a well-established fibrosis marker in patients with CLD.<sup>[18,23–26]</sup> However, the relationship between serum HA level and PEM in patients with CLD remains unclear. As mentioned earlier, PEM is linked to high morbidity and mortality

**Table 3**

**Correlation with serum HA level in each variable.**

<b>Correlation with HA level (all cases, n=298)</b>			<b>Correlation with HA level (LC, n=164)</b>		
	<b>r<sub>s</sub></b>	<b>P value</b>		<b>r<sub>s</sub></b>	<b>P value</b>
Age	0.417	<0.0001	Age	0.125	0.1096
WBC	-0.392	<0.0001	WBC	-0.260	0.0008
Lymphocyte count	-0.433	<0.0001	Lymphocyte count	-0.295	0.0001
AST	0.483	<0.0001	AST	0.232	0.0029
ALT	0.146	0.0115	ALT	0.007	0.9295
ALP	0.569	<0.0001	ALP	0.394	<0.0001
GGT	0.251	<0.0001	GGT	0.155	0.0473
Total bilirubin	0.385	<0.0001	Total bilirubin	0.435	<0.0001
Serum albumin	-0.661	<0.0001	Serum albumin	-0.577	<0.0001
Platelet count	-0.685	<0.0001	Platelet count	-0.421	<0.0001
PT	-0.667	<0.0001	PT	-0.564	<0.0001
Total cholesterol	-0.417	<0.0001	Total cholesterol	-0.354	<0.0001
Triglyceride	-0.149	0.0108	Triglyceride	-0.181	0.0209
REE	-0.075	0.1988	REE	-0.0805	0.3054
REE/BW	-0.121	0.0376	REE/BW	-0.079	0.3166
BMI	0.127	0.0285	BMI	0.078	0.3244
Ferritin (n=292)	-0.025	0.6673	Ferritin (n=160)	-0.016	0.8453
hCRP (n=230)	0.252	0.0001	hCRP (n=120)	0.215	0.0183
Free fatty acid (n=230)	0.304	<0.0001	Free fatty acid (n=120)	0.354	<0.0001

hCRP and free fatty acid were tested using stored sera. ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, BW = body weight, GGT = gamma glutamyl transpeptidase, HA = hyaluronic acid, hCRP = high-sensitivity C reactive protein, LC = liver cirrhosis, PT = prothrombin time, REE = rest energy expenditure, WBC = white blood cells.

**Table 4****Univariate and multivariate analyses of factors linked to PEM.**

Variables	Univariate	Multivariate analysis		
	P value	Odds ratio	95% CI	P value
Age, y	0.0009	0.970*	0.910–1.031	0.3334
Gender, female/male	0.8607			
HCV-RNA $\geq 5$ log copies/mL, yes/no	0.2192			
AST, IU/L	<0.0001	0.994*	0.973–1.016	0.5759
ALT, IU/L	0.6352			
ALP, IU/L	<0.0001	0.999*	0.998–1.001	0.9691
GGT, IU/L	0.3054			
Total cholesterol, mg/dL	<0.0001	1.010*	0.994–1.027	0.2308
Triglyceride, mg/dL	0.0043	1.000*	0.991–1.014	0.9586
Total bilirubin, mg/dL	<0.0001	1.124*	0.596–2.220	0.7234
White blood cell, per mm <sup>3</sup>	0.0006	0.999*	0.998–1.001	0.9611
Lymphocyte count, per mm <sup>3</sup>	0.0003	1.001*	0.999–1.003	0.7757
Platelet count, $\times 10^4/\text{mm}^3$	<0.0001	0.983*	0.847–1.165	0.8292
Prothrombin time, %	<0.0001	1.046*	1.003–1.091	0.0351
Serum hyaluronic acid, ng/mL	<0.0001	0.996*	0.994–0.998	0.0001
APRI	<0.0001	0.951*	0.855–1.048	0.3166
FIB-4 index	<0.0001	1.016*	0.983–1.031	0.3655
AST to ALT ratio	<0.0001	0.494*	0.135–1.730	0.2719
Forns index	<0.0001	1.001*	0.522–1.949	0.9969
Body mass index, kg/m <sup>2</sup>	0.0456	1.001*	0.902–1.115	0.9789
REE, kcal/d	0.2714			

ALP=alkaline phosphatase, ALT=alanine aminotransferase, APRI=AST to platelet ratio index, AST=aspartate aminotransferase, CI=confidence interval, GGT=gamma glutamyl transpeptidase, HCV=hepatitis C virus, PEM=protein-energy malnutrition, REE=rest energy expenditure.

\* Hazard ratio for 1 unit in continuous variables.

in patients with LC. Thus identifying factors closely associated with PEM are essential for clinicians. Different liver diseases can cause different patterns of liver fibrogenesis. Based on recent reports, there are several controversial results related to the clinical applicability of serum HA level in various liver diseases, including hepatitis B or C, autoimmune liver disease, alcoholic liver disease, NASH, and others.<sup>[18,19]</sup> Thus, in the current study, we investigated the effect of serum HA level in limited patients with CHC, who are most common among Japanese patients with CLD. To the best of our knowledge, this is the 1st study examining the relationship between serum HA level and PEM as defined by using indirect calorimetry for patients with CHC.

In our results, the AUROC of serum HA level for the presence of PEM was the highest among those of the 6 serum fibrosis markers for all cases (AUROC=0.849) and for patients with LC (AUROC=0.771), although APRI had the highest AUROC (0.854) for the presence of PEM in patients with non-LC. In the limited patients whose stored sera were available, similar results were obtained. Furthermore, the AUROC of HA level for predicting LC was 0.879 in our analysis and HA level was found to be a significant factor linked to PEM in the multivariate analysis. These results suggest that serum HA level is a useful predictor of not only LC but also PEM. In daily clinical practice, testing serum HA level can be recommended for evaluating PEM in patients with CLD as assessment of HA is an inexpensive, standardized, and noninvasive supplement although indirect calorimetry is expensive and time consuming for testing. Serum HA level may also be a useful indicator for initiating nutritional support for patients with PEM.

On the other hand, in our results, serum HA level was significantly correlated with numerous factors including age, biliary enzymes, liver function, immunological function as expressed by WBC and lymphocyte count, and systemic

inflammation as expressed by hCRP value. Thus, in a sense, our observations that HA level is a significant predictor for PEM may be associated with complex factors. However, it worth noting that hCRP level was significantly correlated with HA level in the present analysis ( $r_s=0.252$ ,  $P=0.0001$ ) although there are several missing values for hCRP. A is an immune regulator that acts through the release of inflammatory cytokines and it is produced by fibroblasts.<sup>[21,42]</sup> In inflammatory diseases, fibroblasts are activated in the repair process of inflammation, and thus the production of HA is considered to be enhanced when the failed component is repaired.<sup>[21,42]</sup> Based on these, we speculate that a higher HA level is linked to systemic inflammation, which eventually leads to energy consumption or malnutrition, as well as protein malnutrition.<sup>[42,43]</sup> However, further examination will be needed to confirm these results.

Hanai et al<sup>[41]</sup> reported that plasma levels of FFA were significantly correlated with npRQ value ( $n=146$ ,  $r=-0.39$ ,  $P<0.0001$ ) and FFA is a useful alternative marker to represent npRQ in patients with LC, whereas in our data, in patients with LC (120 patients with available stored sera), although significant correlation was found between FFA level and serum HA level ( $r_s=0.354$ ,  $P<0.0001$ ), no significant correlation was found between FFA level and npRQ ( $r_s=-0.113$ ,  $P=0.219$ ). Thus whether serum FFA value significantly correlates with npRQ remains controversial, and this is beyond the aim of the present analysis.

We acknowledge several limitations to the present study. First, this is a retrospective observational study. Second, liver biopsy involves a drawback being prone to sampling errors for evaluating the degree of liver fibrosis. Third, there are several missing values for testing several variables. Fourth, npRQ value may be influenced by characteristics of diet or recent physical activity in each patient, leading to bias. Thus caution should be exercised in interpreting our study results. However, in the

current analysis, we demonstrated that serum HA level was closely associated with PEM in patients with CHC. In conclusion, serum HA level can be a useful predictor for predicting PEM in patients with CHC.

## Acknowledgments

The authors would like to thank all medical staff in our nutritional guidance room for data collection.

## References

- [1] Charlton MR. Branched-chain amino acid enriched supplements as therapy for liver disease. *J Nutr* 2006;136(suppl):295S–8S.
- [2] Moriwaki H, Miwa Y, Tajika M, et al. Branched-chain amino acids as a protein- and energy-source in liver cirrhosis. *Biochem Biophys Res Commun* 2004;313:405–9.
- [3] Moctezuma-Velázquez C, García-Juárez I, Soto-Solís R, et al. Nutritional assessment and treatment of patients with liver cirrhosis. *Nutrition* 2013;29:1279–85.
- [4] Nusrat S, Khan MS, Fazili J, et al. Cirrhosis and its complications: evidence based treatment. *World J Gastroenterol* 2014;20:5442–60.
- [5] Kawaguchi T, Izumi N, Charlton MR, et al. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. *Hepatology* 2011;54:1063–70.
- [6] Periyalwar P, Dasarathy S. Malnutrition in cirrhosis: contribution and consequences of sarcopenia on metabolic and clinical responses. *Clin Liver Dis* 2012;16:95–131.
- [7] Montano-Loza AJ. Clinical relevance of sarcopenia in patients with cirrhosis. *World J Gastroenterol* 2014;20:8061–71.
- [8] Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014;383:1749–61.
- [9] Plauth M, Cabré E, Campillo B, et al. ESPEN guidelines on parenteral nutrition: hepatology. *Clin Nutr* 2009;28:436.
- [10] Terakura Y, Shiraki M, Nishimura K, et al. Indirect calorimetry and anthropometry to estimate energy metabolism in patients with liver cirrhosis. *J Nutr Sci Vitaminol (Tokyo)* 2010;56:372–9.
- [11] Peng S, Plank LD, McCall JL, et al. Body composition, muscle function, and energy expenditure in patients with liver cirrhosis: a comprehensive study. *Am J Clin Nutr* 2007;85:1257–66.
- [12] Sam J, Nguyen GC. Protein-calorie malnutrition as a prognostic indicator of mortality among patients hospitalized with cirrhosis and portal hypertension. *Liver Int* 2009;29:1396–402.
- [13] Huisman EJ, Trip EJ, Siersema PD, et al. Protein energy malnutrition predicts complications in liver cirrhosis. *Eur J Gastroenterol Hepatol* 2011;23:982.
- [14] Bota S, Herkner H, Sporea I, et al. Meta-analysis: ARFI elastography versus transient elastography for the evaluation of liver fibrosis. *Liver Int* 2013;33:1138–47.
- [15] Sporea I, Gilja OH, Bota S, et al. Liver elastography—an update. *Med Ultrason* 2013;15:304–14.
- [16] Colli A, Fraquelli M, Casazza G, et al. The architecture of diagnostic research: from bench to bedside—research guidelines using liver stiffness as an example. *Hepatology* 2014;60:408–18.
- [17] Singh S, Fujii LL, Murad MH, et al. Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013;11:1573–84.
- [18] Rostami S, Parsian H. Hyaluronic acid: from biochemical characteristics to its clinical translation in assessment of liver fibrosis. *Hepat Mon* 2013;13:e13787.
- [19] Necas J, Bartosikova L, Brauner P, et al. Hyaluronic acid (hyaluronan): a review. *Vet Med* 2008;53:397–411.
- [20] Rossi E, Adams LA, Ching HL, et al. High biological variation of serum hyaluronic acid and Hepascore, a biochemical marker model for the prediction of liver fibrosis. *Clin Chem Lab Med* 2013;51:1107–4.
- [21] Eriksson S, Fraser JR, Laurent TC, et al. Endothelial cells are a site of uptake and degradation of hyaluronic acid in the liver. *Exp Cell Res* 1983;144:223–8.
- [22] Wong CS, Gibson PR. Effects of eating on plasma hyaluronan in patients with cirrhosis: its mechanism and influence on clinical interpretation. *J Gastroenterol Hepatol* 1998;13:1218–4.
- [23] Pares A, Deulofeu R, Gimenez A, et al. Serum hyaluronate reflects hepatic fibrogenesis in alcoholic liver disease and is useful as a marker of fibrosis. *Hepatology* 1996;24:1399–403.
- [24] Zhang YX, Wu WJ, Zhang YZ, et al. Noninvasive assessment of liver fibrosis with combined serum aminotransferase/platelet ratio index and hyaluronic acid in patients with chronic hepatitis B. *World J Gastroenterol* 2008;14:7117–21.
- [25] Suzuki A, Angulo P, Lymp J, et al. Hyaluronic acid, an accurate serum marker for severe hepatic fibrosis in patients with non-alcoholic fatty liver disease. *Liver Int* 2005;25:779–86.
- [26] Halfon P, Bourliere M, Penaranda G, et al. Accuracy of hyaluronic acid level for predicting liver fibrosis stages in patients with hepatitis C virus. *Comp Hepatol* 2005;4:6.
- [27] Valva P, Casciato P, Diaz Carrasco JM, et al. The role of serum biomarkers in predicting fibrosis progression in pediatric and adult hepatitis C virus chronic infection. *PLoS ONE* 2011;6:e23218.
- [28] Patel K, Remlinger KS, Walker TG, et al. Multiplex protein analysis to determine fibrosis stage and progression in patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2014;12:2113–0. e1–3.
- [29] Fernandes FF, Ferraz ML, Andrade LE, et al. Enhanced liver fibrosis panel as a predictor of liver fibrosis in chronic hepatitis C patients. *J Clin Gastroenterol* 2015;49:235–41.
- [30] Lichtinghagen R, Pietsch D, Bantel H, et al. The enhanced liver fibrosis (ELF) score: normal values, influence factors and proposed cut-off values. *J Hepatol* 2013;59:236–42.
- [31] Tajika M, Kato M, Mohri H, et al. Prognostic value of energy metabolism in patients with viral liver cirrhosis. *Nutrition* 2002;18:229–34.
- [32] Shiraki M, Nishiguchi S, Saito M, et al. Nutritional status and quality of life in current patients with liver cirrhosis as assessed in 2007–2011. *Hepatol Res* 2013;43:106–2.
- [33] Wai CT, Greenon JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003;38:518–26.
- [34] Sterling RK, Lissen E, Clumeck N, et al. APRICOT Clinical Investigators: development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006;43:1317–25.
- [35] Forns X, Ampurdanès S, Llovet JM, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002;36(pt 1):986–2.
- [36] The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994;20(part 1):15–20.
- [37] Iwata K, Enomoto H, Nishiguchi S, et al. Serum zinc value in patients with hepatitis virus-related chronic liver disease: association with the histological degree of liver fibrosis and with the severity of varices in compensated cirrhosis. *J Clin Biochem Nutr* 2014;55:147–52.
- [38] Dickerson RN, Tidwell AC, Minard G, et al. Predicting total urinary nitrogen excretion from urinary urea nitrogen excretion in multiple-trauma patients receiving specialized nutritional support. *Nutrition* 2005;21:332–8.
- [39] Teramoto A, Yamanaka-Okumura H, Urano E, et al. Comparison of measured and predicted energy expenditure in patients with liver cirrhosis. *Asia Pac J Clin Nutr* 2014;23:197–204.
- [40] Kato M, Miwa Y, Tajika M, et al. Preferential use of branched-chain amino acids as an energy substrate in patients with liver cirrhosis. *Intern Med* 1998;37:429–34.
- [41] Hanai T, Shiraki M, Nishimura K, et al. Free fatty acid as a marker of energy malnutrition in liver cirrhosis. *Hepatol Res* 2014;44:218.
- [42] Jiang D, Liang J, Noble PW. Hyaluran as an immune regulator in human diseases. *Physiol Rev* 2011;91:221–64.
- [43] Preidis GA, Keaton MA, Campeau PM, et al. The undernourished neonatal mouse metabolome reveals evidence of liver and biliary dysfunction, inflammation, and oxidative stress. *J Nutr* 2014;144:273–81.